TO STUDY THE IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA 1 IN BREAST CANCER AND TO ASSESS ITS ASSOCIATION WITH EXPRESSION OF ER, PR AND HER 2 RECEPTORS

A thesis submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY, CHENNAI

in partial fulfillment of the requirements for the award of the degree of

M.D in PATHOLOGY

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCE & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

CERTIFICATE

This is to certify that the dissertation work entitled **"TO STUDY THE IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA 1 IN BREAST CANCER AND TO ASSESS ITS ASSOCIATION WITH EXPRESSION OF ER, PR AND HER 2 RECEPTORS"** submitted by **Dr K.S.Karthikeyan,** is a work done by him during the post graduate period of study in the Department of Pathology. This work was done under the guidance of **Dr S.Shanthakumari,** Professor, Department of Pathology, PSG IMS&R.

Dr. Prasanna N Kumar
Professor & HOD, Pathology
PSGIMS & R
Coimbatore – 04

Dr.S.Ramalingam
Dean
PSGIMS & R
Coimbatore – 04

CERTIFICATE

This is to certify that the thesis entitled **"TO STUDY THE IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA 1 IN BREAST CANCER AND TO ASSESS ITS ASSOCIATION WITH EXPRESSION OF ER, PR AND HER 2 RECEPTORS"** submitted by **Dr K.S.Karthikeyan** to The Tamilnadu Dr MGR Medical University, Chennai, for the award of the degree of **Doctor of Medicine in Pathology,** is a bonafide record of research work carried out by him under my guidance. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Coimbatore – 641004

Dr S.Shanthakumari
Professor of Pathology
PSG IMS&R
Coimbatore - 641004

DECLARATION

I, **Dr.K.S.Karthikeyan**, do hereby declare that the thesis entitled **"TO STUDY THE IMMUNOHISTOCHEMICAL EXPRESSIONOF BRCA 1 IN BREAST CANCER AND TO ASSESS ITS ASSOCIATION WITH EXPRESSION OF ER, PR AND HER 2 RECEPTORS"** is a bonafide work done by me under the guidance of **Dr S.Shanthakumari,** Professor in the Department of Pathology, PSG Institute of Medical Sciences & Research. This study was performed at the PSG Institute of Medical Sciences & Research, Coimbatore, under the aegis of the The Tamilnadu Dr MGR Medical University, Chennai, as part of the requirement for the award of the MD degree in Pathology.

Coimbatore – 641004

Dr K.S.Karthikeyan
MD (Pathology) postgraduate
Department of Pathology
PSGIMS&R
Coimbatore

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

May 19, 2014

To
Dr K S Karthikeyan
Postgraduate
Department of Pathology
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 9th May, 2014 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your study proposal entitled:

"To study the immunohistochemical expression of BRCA-1 protein in breast cancer and to assess its association with expression of Estrogen Receptors, Progesterone Receptors and Her2 NEU"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Confidentiality statement
4. Application for waiver of consent
5. Data collection tool
6. CV
7. Budget

After due consideration, the Committee has decided to approve the study.

The members who attended the meeting at which your study proposal was discussed are as follows:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr Y S Sivan	Ph D	Member –Social Scientist	Male	Yes	Yes

The approval is valid for one year.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

19.5.17

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

ACKNOWLEDGEMENTS

This thesis has been accomplished by me with the help and support of many people.

At first let me express my sincere devotions to Almighty for His Divine Inspiration

I must give my high, respectful gratitude to my mentor **Professor Dr. S. Shanthakumari, Department of Pathology**, **PSG IMS&R,** for her guidance, supervision and help throughout this project. I have learned a lot throughout the study with many challenges yet gained valuable experiences in order to complete the task.

I wish to express my sincere thanks and gratitude to **Dr. S. Ramalingam, Dean, PSG IMS & R** for providing the facilities to conduct this work in this institution.

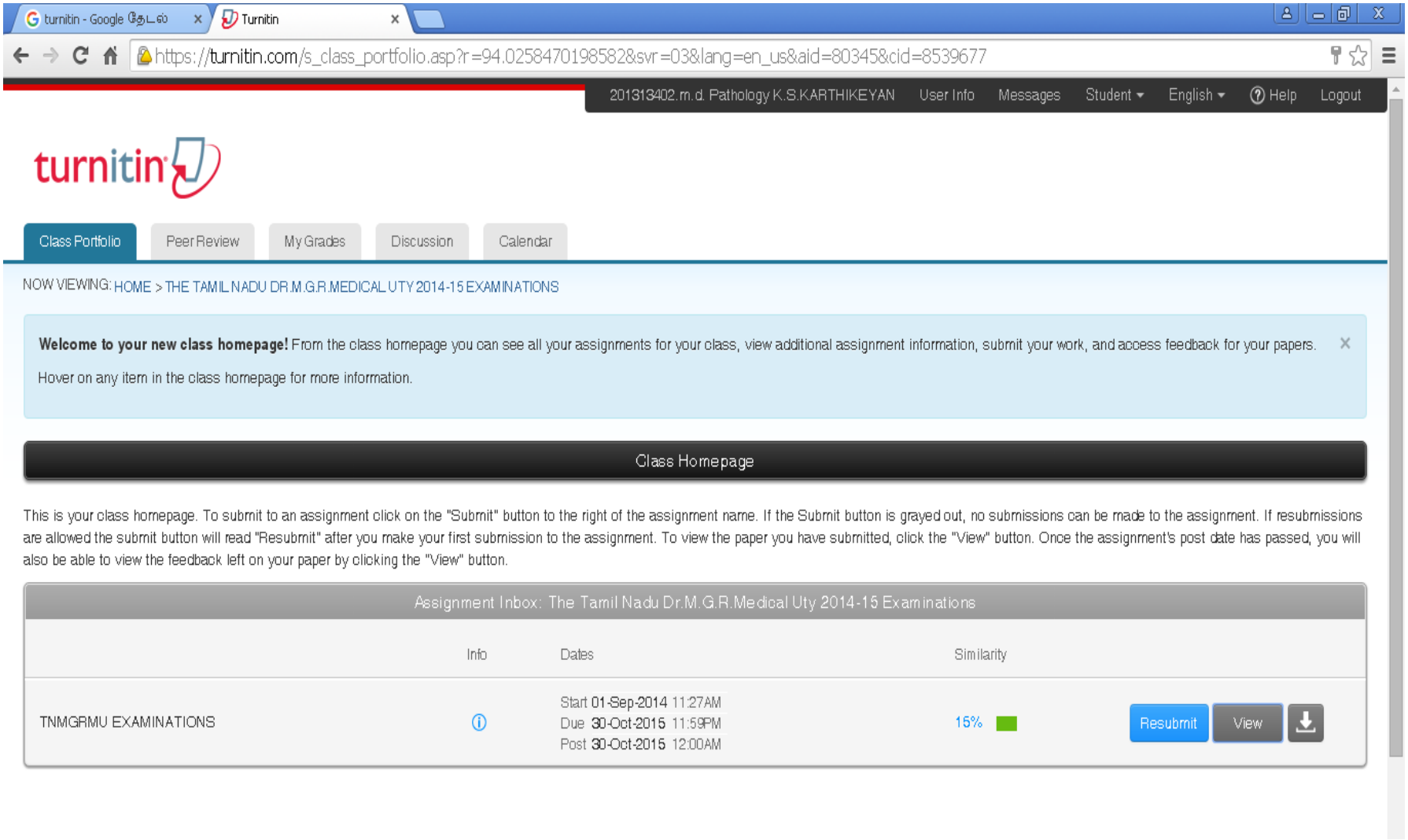
I render my gratefulness and sincere thanks to **Dr. Prasanna N Kumar, Professor and HOD of Pathology and Dr T M Subbarao, Professor of Pathology** for providing me with a much needed motivation and guidance which made me to improve myself as a better person.

I express my thanks to all the technicians, particularly to Angeline Mary who helped me in every way she could and other staffs in the

department of Pathology, PSG IMS & R, Coimbatore, who have helped me in this study.

Special thanks to **Dr. Suma B Pillai**, professor, department of pathology, PSG IMS& R, who has motivated me to conduct this study and to my dear colleagues Dr. Sharanya and Dr. Shuba who were with me during every thick and thin time and provided me with great support than I could have ever imagined.

Finally I would like to express my eternal appreciation towards my parents who have always been there for me no matter where I am, for all unconditional support and patience.


turnitin - Google தேடல்
Turnitin
https://turnitin.com/s_class_portfolio.asp?r=94.0258470198582&svr=03&lang=en_us&aid=80345&cid=8539677
201313402.m.d. Pathology K.S.KARTHIKEYAN
User Info
Messages
Student
English
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2014-15 EXAMINATIONS
Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations
Info
Dates
Similarity
TNMGRMU EXAMINATIONS
Start 01-Sep-2014 11:27AM
Due 30-Oct-2015 11:59PM
Post 30-Oct-2015 12:00AM
15%
Resubmit
View

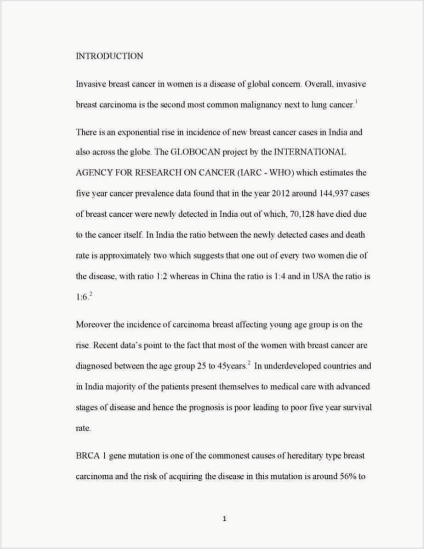
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	201313402.m.d. Pathology K.S.KAR..
Assignment title:	TNMGRMU EXAMINATIONS
Submission title:	TO STUDY THE IMMUNOHISTOCH..
File name:	3.final_full_thesis_-_correction_pe ..
File size:	330.5K
Page count:	85
Word count:	12,910
Character count:	70,097
Submission date:	25-Sep-2015 08:45PM
Submission ID:	574792914



INTRODUCTION

Invasive breast cancer in women is a disease of global concern. Overall, invasive breast carcinoma is the second most common malignancy next to lung cancer.[1]

There is an exponential rise in incidence of new breast cancer cases in India and also across the globe. The GLOBOCAN project by the INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC - WHO) which estimates the five year cancer prevalence data found that in the year 2012 around 144,937 cases of breast cancer were newly detected in India out of which, 70,128 have died due to the cancer itself. In India the ratio between the newly detected cases and death rate is approximately two which suggests that one out of every two women die of the disease, with ratio 1:2 whereas in China the ratio is 1:4 and in USA the ratio is 1:6.[2]

Moreover the incidence of carcinoma breast affecting young age group is on the rise. Recent data's point to the fact that most of the women with breast cancer are diagnosed between the age group 25 to 45years.[2] In underdeveloped countries and in India majority of the patients present themselves to medical care with advanced stages of disease and hence the prognosis is poor leading to poor five year survival rate.

BRCA 1 gene mutation is one of the commonest causes of hereditary type breast carcinoma and the risk of acquiring the disease in this mutation is around 56% to

1

TABLE OF CONTENTS

TO STUDY THE IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA 1 IN BREAST CANCER AND TO ASSESS ITS ASSOCIATION WITH EXPRESSION OF ER, PR AND HER 2RECEPTORS

Invasive breast cancer in women is a disease of global concern. Overall, invasive breast carcinoma is the second most common malignancy next to lung cancer.

BRCA 1 gene mutation is one of the commonest causes of hereditary type breast carcinoma. BRCA 1 mutation if detected earlier, would improve the prognosis of patient in terms of devising a treatment plan that involves prophylactic mastectomy as these patients are prone to develop carcinoma sooner or later. Therefore mutational studies might help in reducing the rate of incidence of breast carcinoma.

Therefore we propose to study the prevalence of BRCA 1 mutation in breast carcinoma by Immunohistochemical study, its association with various phenotypic features and hormonal receptors

This is a retrospective study. Cases reported as carcinoma breast were retrieved from the archives of department of pathology, between the years 2012 to 2015. H&E, IHC staining with ER, PR,HER 2 is done for the 42 selected cases and correlated with each other.

Finally our study on breast carcinoma specimen revealed the incidence is around 3.1% of breast cancer among women in our institute. Majority of which occurs in the mean age group of 50.5 years. We infer that BRCA 1 mutation was seen most in grade 3 / high grade tumors. BRCA 1 mutation was also seen in hormone receptor negative cases, in particular estrogen receptors. The limitations of our study include small sample size. A larger prospective study on the utility of BRCA 1immunohistochemistry is essential to prove the benefits of this mutation analysis for routine treatment and prognostic reasons.

KEYWORDS: Breast carcinoma, BRCA 1, ER, PR, HER 2, Immunohistochemistry.

INTRODUCTION

Invasive breast cancer in women is a disease of global concern. Overall, invasive breast carcinoma is the second most common malignancy next to lung cancer.[1]

There is an exponential rise in incidence of new breast cancer cases in India and also across the globe. The GLOBOCAN project by the INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC - WHO) which estimates the five year cancer prevalence data found that in the year 2012 around 144,937 cases of breast cancer were newly detected in India out of which, 70,128 have died due to the cancer itself. In India the ratio between the newly detected cases and death rate is approximately two which suggests that one out of every two women die of the disease, with ratio 1:2 whereas in China the ratio is 1:4 and in USA the ratio is 1:6.[2]

Moreover the incidence of carcinoma breast affecting young age group is on the rise. Recent data's point to the fact that most of the women with breast cancer are diagnosed between the age group 25 to 45years.[2] In underdeveloped countries and in India majority of the patients present themselves to medical care with advanced stages of disease and hence the prognosis is poor leading to poor five year survival rate.

BRCA 1 gene mutation is one of the commonest causes of hereditary type breast carcinoma and the risk of acquiring the disease in this mutation is around 56% to 90%.[3,4] BRCA 1 mutation if detected earlier, would improve the prognosis of patient in terms of devising a treatment plan that involves prophylactic mastectomy as these patients are prone to develop carcinoma sooner or later. [5] Therefore mutational studies might help in reducing the rate of incidence of breast carcinoma.

The best known method for detection of BRCA 1 mutation is by molecular analysis, which is time consuming and expensive. But now due to the advent of Immunohistochemical studies it has become easier to detect the mutation, which is cost effective, less cumbersome and results are provided sooner.

Therefore we propose to study the prevalence of BRCA 1 mutation in breast carcinoma by Immunohistochemical study, its association with various phenotypic features and hormonal receptors.

.

AIMS AND OBJECTIVES

(i) To study the immunohistochemical expression of BRCA1 protein in carcinoma breast and

(ii) To correlate its expression with the phenotype and receptor status - estrogen receptor, progesterone receptor and HER 2 receptor

REVIEW OF LITERATURE

EMBRYOLOGY:

Breast develops along milk line or mammary ridges that extend from axilla to groin. By 5th week of gestation, there occurs thickening of ectoderm in the ventral aspect of the embryo forming milk line.[6] Normally this line is resolved except for a portion, which is remnant over the pectoral area and develops into breast. In this region mammary gland develops, at first a thick mass of epidermal cells grows downward into the mesenchyme. From this mass 15 to 20 projections arise that extends into the mesenchyme. These projections are then canalized. Proximal part of the projections is converted into major ducts called lactiferous ducts. The terminal portion of the projection proliferates and forms the secretory unit which later develops into terminal duct lobular unit. Lactiferous ducts initially open into a pit formed by the thickened epidermal cells. As the underlying mesenchyme grows it pushes the pit out and forms an elevation above the surface which is called the nipple. In women as the age progress these ducts and secretory unit undergo drastic changes in particular during puberty and pregnancy.[7]

GROSS ANATOMY OF BREAST:

There are many factors which influence the size and shape of the breast, these includes genetic variations, ethnicity, food habits, age, menstrual status, parity etc.

Anatomical location of the mature breast can be roughly demarcated in vertical plane and transverse plane. In vertical plane breast is situated from second to sixth portion of rib and in transverse plane from the edge of the sternum along its medial side to axillary line which is taken as the lateral extension of breast.[8]

Breast is covered by skin and subcutaneous tissue that overlies deep pectoral fascia, the structures that are found beneath this deep pectoral fascia are few groups of muscles, located superiorly and inferiorly. Superior muscles are pectoralis major and serratus anterior whereas inferior muscle is external oblique. A potential space called submammary space which denotes the space developed between deep fascia and breast tissue is made up of loose connective tissue which allows the breast to be mobile.[8]

Nipple is located roughly corresponding to the fourth intercostal space at the centre along midclavicular line. The skin overlying and surrounding the nipple is called areola. They contain sweat glands and sebaceous glands that have a direct communication with skin externally. These glands function to produce an oily secretion which reduces the friction in the nipple areolar complex that is caused when the neonate starts to feed. Hence the glands present here appear as an elevation surrounding the areola, a characteristic finding in feeding mothers and are called as Montgomery tubercles. Owing to the presence of considerable amount of melanocytes in the nipple areolar complex, they appear dark in colour.[6]

FUNCTIONAL ANATOMY OF BREAST:

Breast is comprised of approximately 15 to 25 lobes.[6] These lobes are composed of large ducts, its branches and terminal duct lobular units(TDLU). TDLU communicates with nipple through larger ducts called lactiferous ducts. A lactiferous duct undergoes branching to form segmental duct, segmental duct in turn branches into sub segmental ducts and these ducts further divide into smaller terminal ducts. Terminal ducts finally end with small acini to form a lobule, called as terminal duct lobular unit. Many such terminal duct lobular unit forms one lobe. Terminal duct lobular unit is considered to have a secretory function.[9] Intervening stroma between two lobes is known as interlobular stroma were as stroma in between two terminal duct lobular unit is called as intralobularstroma.

Breast changes are more evident in the reproductive age group of women and also during pregnancy in relation to the changes in the hormonal levels. Estrogen and rising progesterone levels during menstruation cause increase in the number of acini per lobule. At the time of pregnancy the number and size of lobules increase exponentially. Lobules play a critical role in production of colostrums after birth and converting into milk. At the time of menopause, these lobules tend to regress in size considerably and stroma is converted into radio dense fibrous stroma.[1] Histologically breast tissue is composed of ducts and stroma. The ducts are lined by bi-layered epithelium of luminal columnar cells and abluminal myoepithelial

cells. Nipple areolar complex and skin covering the breast is lined by keratinizing squamous epithelium.

BREAST CANCER:

WHO defines invasive breast cancer as a group of malignant epithelial tumours which is characterized by invasion into adjacent tissues, lymph nodes and increased tendency to metastasize to distant sites. Most of the breast tumors belong to the group of adenocarcinoma and tend to arise from the breast parenchymal epithelium particularly from that of the terminal duct lobular unit.[10]

Carcinoma breast is the commonest non skin malignant tumor and accounts for about 22% of all malignancies in women worldwide. Since the year 2008, according to the International Agency for Research on Cancer, the incidence of breast cancer has considerably increased about 20% and mortality has increased by about 14%. It estimates that out of every four women affected with cancer one tends to have breast cancer.[2]

For the last two decades cancer cervix was the primary cause of death among all the cancers in women, in India. However, now breast cancer is considered as the most common cause of cancer deaths in women. This is pertaining to the fact that in western and other developed countries five year survival rate has been good because of early detection of breast cancer due to better awareness about the disease and implementation of effective screening programs. Whereas in

underdeveloped countries, as well as in India most of the patients present themselves to medical care in the terminal stage of the disease where the prognosis is poor, hence there is a substantial increase in mortality rate.

Incidence curve for breast cancer rises around puberty and peaks at menopause and thereafter maintains a steady state.[9] The incidence of breast cancer affecting young age group is on the rise. Now most of the women with breast cancer are diagnosed between the age group 25 – 45years.[2]

According to statistics provided by cancer institute (W.I.A), Chennai between the year 2006 and 2008 breast cancer accounts for about 26.3% of all cancers in females. This shows the heavy burden of disease within a regional population.[11]

EPIDEMIOLOGY:

Breast cancer is regarded as a disease of affluent society denoting to the fact that modified lifestyle leads to intake of high caloric diet and less amount of physical activity, as both are considered a major risk factor for the disease.[11]

1. RACE:

It varies markedly with different ethnic groups because each group may have subtle variations in the breast cancer genes leading to the difference in incidence

for each country. As example BRCA 1 and BRCA 2 mutations are most common among the Jews.[12]

2. AGE GROUP:

Earlier it was said breast cancer was disease of old, but recent trends show that the common age group being affected are from 25-45 years of age.[2]

3. MENSTURAL AND REPRODUCTIVE HISTORY:

Early menarche, less than 11 years of age and late menopause is viewed as a risk factor for breast cancer. Delivering first child at full term around the age of 20 years generally declines the risk of obtaining breast cancer by 50% than that of women who were nulliparous or who delivers their first child at an age of 30 years or more.[13]

4. ROLE OF ESTROGEN:

Increased levels of estrogen is one of the most important concerning factor in breast cancer, mostly in the form of post menopausal hormone treatment which is an exogenous form of estrogen administration and majority of the breast carcinoma cases detected are estrogen receptor positive.[14] The national toxicology program, in year 2002 declared estrogen a carcinogenic agent. Endogenous production of estrogen can be reduced by doing oophorectomy which tends to reduce the risk of acquiring breast cancer by 75%.[1]

5. NUTRITION:

High caloric intake is deemed to be an important risk factor for causing breast cancer. Intake of red meat, alcohol is associated with increased risk. Good amount of physical exercise offers some protection against breast cancer in post menopausal women as they prevent obesity which can cause insulin resistance. Elevated insulin level causes increase in androgen which is then converted into estrogen in the adipose tissue thereby causing breast cancer.[1]

6. DENSITY OF BREAST:

The density of the breast detected by mammogram helps in stratification of individuals with higher risk factors; those with high density have 4 to 6 times[1] increased risk of either estrogen receptor positive or negative breast cancer as than that of those with low densities.

7. RADIATION AND ENVIRONMENT:

Exposure to radiation in the form of therapy or accidental nuclear radiation, leads to an increased rate of breast cancer. Particularly women exposed to radiation at early years of age tend to develop breast cancer because breast is in the development phase at this age group.[15] contamination of surrounding environment withy organophosphorous compounds is also considered as a risk factor for carcinoma breast because it can exhibit an estrogenic effect on humans.[1]

8. LACTATION:

Breast feeding reduces the risk of breast cancer when the duration of feeding is prolonged more than the recommended exclusive six months of breastfeeding.

9. MUTATION:

Tumour suppressor genes play a central role in suppressing the tumour development as far as any tumor is concerned. A specific type of mutation called germ line mutation interfere with the normal functioning of the tumour suppressor genes in breast leading to cancer.[16] Around 90% of women have a risk of developing breast cancer during her lifetime if she was detected to have germline mutation.[1]

10. BREAST CANCER IN FAMILY:

When a woman is found to have breast cancer there is a possibility of about 15-20%[1], that her first degree relatives might have been affected by breast cancer. Highlighting fact is that these individuals do not carry any germline mutations as stated above but the surrounding environmental factors influence the genes that are considered to be low risk for causing breast cancer. Risk is reckoned to be minimal if only mother is the closest relative suffering from breast cancer and it is detected only after her menopause.

PATHOGENESIS:

Breast cancers are generally adenocarcinomas and can be further sub categorized into three types based upon the expression of estrogen and HER2 receptors as, estrogen receptor negative - HER 2 receptor negative; estrogen receptor positive - HER 2 receptor negative; estrogen receptor positive/negative - HER 2 receptor positive.[1] This subcategorization is helpful for clinicians in formulating treatment protocols and thus influencing prognosis. Carcinoma breast in most of the elderly women are positive for estrogen receptor and HER2 receptor; whereas in young women 50% of the cases are found to be estrogen receptor negative.[1]

Cells undergo genetic abnormality when there is an interaction between risk factors, environment and genes that are susceptible, these abnormal cells with chromosomal alterations were cloned and it undergoes proliferation forming a tumor, which is a basic phenomenon of almost all the cancers in human. Causes of breast cancer can be either of two types:

1. Hereditary / Familial breast cancer

2. Sporadic breast cancer

1. HEREDITARY BREAST CANCER / FAMILIAL BREAST CANCER:

When the genes that are predisposed to cause breast cancer are inherited then it is termed familial / hereditary breast cancer. It makes up about 5-10% of all the

breast cancer cases.[17] Risk is increased by several folds when the close first degree relatives are affected like mother, sister.

Few important susceptible genes that were identified as cause to breast cancer are TP53, BRCA1, BRCA2 and CHEK2. The normal function of these tumour suppressor genes are to check cell proliferation and prevent tumour formation by repairing the defective DNA and maintaining genomic stability. These tumour suppressor genes when mutated will lose their normal function and keeps on accumulating the defective gene which leads to development of cancer.

Approximately one tenth of breast cancers can be attributable to familial cause, out of which our gene of study BRCA 1 and also BRCA 2 accounts approximately 90%.[18] Individuals with BRCA 1 gene mutation are also prone to develop ovarian carcinoma (10-20%), epithelial cancers of the prostate and pancreas.[1]

2. SPORADIC MUTATION BREAST CANCER:

The above mentioned risk factors when combined together can cause sporadic mutation resulting in breast cancer. Out of all the risk factors the most significant one is hormone excess, particularly estrogen. Women are generally exposed to excess of hormones in the form of post menopausal hormonal therapy, as a contraceptive or as a part of treatment for dysfunctional uterine bleeding. The number of menstrual cycles attained by a woman in her years has a higher risk of acquiring mutation leading to breast carcinoma. It is due to the fact that during the

menstrual cycle there is an increase in breast epithelial growth. Whenever there is proliferation there is a high chance of damage to DNA which results in accumulation of these damaged DNA. Normally the DNA repair mechanism intervenes to correct the damaged DNA at the end of the menstrual cycle. However, if the repair mechanism fails the mutated DNA tends to settle in the genome. So this is a cyclical process starting from the age of puberty to menopause. Therefore each cycle will have an additive effect of the above mentioned mechanism leading to increased risk of developing breast cancer around the menopausal age group.[1]

WHO HISTOLOGICAL CLASSIFICATION OF TUMORS OF BREAST[10]

EPITHELIAL TUMORS:

Invasive ductal carcinoma, not otherwise specified

Mixed type carcinoma

Pleomorphic carcinoma

Carcinoma with osteoclastic giant cells

Carcinoma with choriocarcinomatous features

Carcinoma with melanotic features

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumours with abundant mucin

Mucinous carcinoma

Cystadenocarcinoma and columnar cell mucinous carcinoma

Signet ring cell carcinoma

Neuroendocrine tumours

Solid neuroendocrine carcinoma

Atypical carcinoid tumour

Small cell / oat cell carcinoma

Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinomas

Pure epithelial metaplastic carcinomas

Squamous cell carcinoma

Adenocarcinoma with spindle cell metaplasia

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Mixed epithelial/mesenchymal metaplastic carcinomas

Lipid-rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen-rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

Lobular carcinoma in situ

Intraductal proliferative lesions

Usual ductal hyperplasia

Flat epithelial atypia

Atypical ductal hyperplasia

Ductal carcinoma in situ

Microinvasive carcinoma

Intraductal papillary neoplasms

Central papilloma

Peripheral papilloma

Atypical papilloma

Intraductal papillary carcinoma

Intracystic papillary carcinoma

Benign epithelial proliferations

Adenosis including variants

Sclerosing adenosis

Apocrine adenosis

Blunt duct adenosis

Microglandularadenosis

Adenomyoepithelialadenosis

Radial scar / complex sclerosing lesion

Adenomas

Tubular adenoma

Lactating adenoma

Apocrine adenoma

Pleomorphic adenoma

Ductal adenoma

MYOEPITHELIAL LESIONS

Myoepitheliosis

Adenomyoepithelialadenosis

Adenomyoepithelioma

Malignant myoepithelioma

MESENCHYMAL TUMOURS

Haemangioma

Angiomatosis

Haemangiopericytoma

Pseudoangiomatous stromal hyperplasia

Myofibroblastoma

Fibromatosis (aggressive)

Inflammatory myofibroblastic tumour

Lipoma

Angiolipoma

Granular cell tumour

Neurofibroma

Schwannoma

Angiosarcoma

Liposarcoma

Rhabdomyosarcoma

Osteosarcoma

Leiomyoma

Leiomyosarcoma

FIBROEPITHELIAL TUMOURS

Fibroadenoma

Phyllodes tumour

Benign

Borderline

Malignant

Periductal stromal sarcoma, low grade

Mammary hamartoma

TUMOURS OF THE NIPPLE

Nipple adenoma

Syringomatous adenoma

Paget disease of the nipple

MALIGNANT LYMPHOMA

Diffuse large B-cell lymphoma

Burkitt lymphoma

Extranodal marginal-zone B-cell lymphoma of MALT type

Follicular lymphoma

METASTATIC TUMOURS

TUMOURS OF THE MALE BREAST

Gynaecomastia

Carcinoma

Invasive

In situ

Breast carcinomas can arise either from ducts or lobules and are called as ductal carcinoma or lobular carcinoma respectively. They are divided into further subsets in which, if the tumor is confined within the ducts or lobules it is known as ductal/lobular carcinoma in situ, if the tumor invades the surrounding stroma it is considered as invasive ductal/lobular carcinoma.

DUCTAL CARCINOMA IN SITU:

The carcinomatous component predominantly arises and is confined within the terminal duct lobular unit. Few of the lesions can arise from larger ducts. They exhibit many morphological patterns such as papillary, solid, cribriform, micropapillary, clinging, cystic hypersecretory and comedo. Further these variants can be either low grade or high grade in nature. Comedo variant is considered as a high grade lesion whereas solid, cribriform, micropapillary variants are low grade lesions. Clinging variant can be of either high grade or lowgrade. A three tier grading systems is being followed, where high grade lesions are classified as grade 3, low grade lesions are classified as grade 1, intermediate lesion are classified as grade 2.[9]

TYPES OF DCIS:

(i) COMEDOCARCINOMA:

It is a high grade, centrally located tumor and can be multicentric at times. Macroscopically they are collection of thickened ducts within the breast tissue that become visible.

Histopathology:

The ducts are composed of tumor cells arranged in solid sheets. The cells exhibit marked pleomorphism with increased mitotic figures. This pattern of carcinoma can also involve the myoepithelial cells. Stroma surrounding the duct

shows a concentric type of fibrosis. Areas of calcification and necrosis are also noted.

Two important aspects to consider in case of DCIS are the extent of involvement of the ducts and for features of invasion. In an invasive breast carcinoma when the DCIS component is more than 25% it is called as Extensive intraductal carcinoma.[19] The main differentiating feature between extensive intraductal and classical invasive carcinomas is that the latter shows irregular invasiveness into the surrounding stroma.

(ii) PAPILLARY CARCINOMA (IN SITU)

It is a rare, low grade lesion commonly seen in the older age group. Macroscopically they appear to be well circumscribed lesion and are larger than papillomas. Histopathologically these neoplasms are composed of cells arranged in a papillary pattern. Cells are uniform in size, with high Nucleo-Cytoplamic ratio, hyperchromatic nuclei and inconspicuous nucleoli. Numerous mitotic figures are also noted. These tumors lack myoepithelial cells and have a scant stroma.

(iii) SOLID FORM OF DCIS:

The ducts are filled by sheets of tumor cells that are larger than cells of lobular neoplasia but small and uniform than that observed in comedocarcinoma

(iv) CRIBRIFORM PATTERN OF DCIS:

The ducts are composed of sheets of tumor cells showing regular spaces interspersed within. When the size, shape and distribution of these spaces are more regular than the lesion is considered to be more malignant. These spaces can be in the form of either trabecular bars or roman bridges. In trabecular bars, there is a perpendicular orientation of nuclei in association with the long axis of the bar. Whereas in roman bridges the trabecular bars appear bent connecting two portions of the epithelial lining.[9]

(v) MICROPAPILLARY VARITEY OF DCIS:

This variant shows tumor cells arranged in a papillary pattern and it lacks central fibrovascular core. They appear as elongated epithelial projections into the lumen.

(vi) CLINGING CARCINOMA:

This variant shows tumor cells arranged in a glandular pattern. Usually they are lined by one to two layers of malignant epithelial cells. These tumor cells are carefully examined because they can exhibit marked pleomorphism and features similar to that of comedocarcinoma. Few of the lesions resemble low grade form of DCIS similar to that of micro papillary variant.

LOBULAR CARCINOMA IN SITU/LOBULAR NEOPLASIA:

Lobular neoplasia accounts for about 1 to 4% of all breast cancer cases, it usually affects women of post-menopausal age group.[10] Most of the lobular carcinomas are multicentric and can be bilateral. Mammogram appears normal except for few cases that show areas of calcification within the central necrosis. Usually it is an incidental finding in breast specimen that has been operated for some other lesion. Macroscopically there are no appreciable features.

Histopathology:

Lobular carcinoma in situ arises within terminal duct lobular unit with pagetoid involvement of the terminal ducts. The neoplasm is composed of monomorphic small round cells with uniform nuclei, fine chromatin and inconspicuous nucleoli. These cells expand the acini within the lobule but however the lobular architecture is preserved. Myoepithelial cells can also be identified in few cases where they are interspersed within the tumor cells.

Lobular carcinoma in situ can be of two types namely[10]

(i)Type A and

(ii)Type B.

Type A has a classical appearance as mentioned above were as type B shows pleomorphic nuclei with condensed chromatin and occasional conspicuous

nucleoli. Type B is also known as pleomorphic lobular neoplasia. If there is Pagetoid involvement, then they show a characteristic clover leaf or necklace appearance (who). When the terminal ducts near the lesion show features similar to that of those seen in the acini then it is called as pagetoid appearance.

Lobular carcinoma in situ can be confused with lobular hyperplasia in which the latter shows smaller sized lobules with a central lumen. The risk of lobular carcinoma in situ developing into invasive cancer is around 20-30%.[9] The risk of developing invasive carcinoma in the normal contralateral breast is also high. Hence lifelong periodical follow up is necessary.

MICROINVASIVE CARCINOMA:

It accounts for less than one percent of all breast carcinoma cases. They are usually less than or equal to one mm in size. Microscopically they show only tiny foci of invasion into the stroma. Rest of the tumor is noninvasive in nature.

INVASIVE DUCTAL CARCINOMA, NOT OTHERWISE SPECIFIED:

This type accounts for the largest group of invasive cancer. These carcinomas have no characteristic morphological appearance as that of tubular or lobular carcinoma. Breast carcinomas due to BRCA 1 mutation are mostly invasive ductal carcinoma, NOS in type.[20]

Macroscopically these tumors have ill-defined margins with stellate outlines, firm in consistency and cut surface appears gritty with yellow specs.

Histopathologically tumor cells are arranged in tubules, cords, clusters and sheets. Cells are large with eosinophilic cytoplasm, pleomorphic nuclei, fine to coarse chromatin and prominent often multiple nucleoli. Variable mitosis are also seen. Most of the invasive carcinoma are associated with comedo type of ductal carcinoma in situ. Surrounding stroma show marked fibrosis with areas of sclerosis. Focal necrosis is also noted. Few of the variants of invasive ductal carcinoma not otherwise specified are:

(i)MIXED TYPE CARCINOMA:

For a tumor to be named as invasive ductal carcinoma not otherwise specified, it should have at least more than 50% of non-specialized patterns (who 21). If the non-specialized pattern accounts only for about 10-49% and the rest of them show a recognized special type like tubular or lobular appearance, then it is called as mixed type carcinoma.[10]

(ii)PLEOMORPHIC CARCINOMA:

It is a type of invasive ductal carcinoma NOS, has a very rare occurrence. Most commonly, it occurs in people around the age group of 50 years. They present themselves as a palpable mass and few of the cases manifest themselves first as metastasis. Histopathologically the tumor comprises of highly pleomorphic tumor giant cells, which account for about 75% of the tumor and background show features of adenocarcinoma. Numerous mitotic figures are noted, 20/10Hpf.

Lymphovascular invasion can also be observed. Axillary node involvement is identified in more than half of the cases.[10]

(iii)CARCINOMA WITH OSTEOCLASTIC GIANT CELLS:

The tumor shows a well to moderately differentiated infiltrating ductal carcinoma, with surrounding stroma exhibiting fibroblastic reaction and increased inflammatory infiltrate of lymphocytes, monocytes and a few macrophages. Characteristic osteoclastic type giant cells are in the stroma that appears to surround the carcinomatous component along with hypervascular reactive stroma is also noted.

(iv)CARCINOMA WITH CHORIOCARCINOMATOUS FEATURES:

Most of the patients with ductal carcinoma NOS have elevated beta chorionic gonadotropin levels in blood. The individual tumor cells are also positive for beta HCG. Choriocarcinomatous differentiation is very rare and it is seen between 5th to 6th decades of life.

(v)CARCINOMA WITH MELANOTIC FEATURES:

These tumors are biphasic and composed of invasive ductal carcinoma component and malignant melanomatous component. It should be differentiated from breast cancers that invade the skin and involve dermo-epidermal junction which leads to deposition of melanin pigment. Most of melanocytic lesions seen within the breast tumor are due to metastasis from malignant melanoma elsewhere.

Primary malignant melanoma involving the nipple areolar complex is extremely rare.

Prognosis of these tumors depends upon the size, grade, vascular invasion and hormonal receptor positivity. Overall ten year survival rate is between 35 and 50%.[21]

INVASIVE LOBULAR CARCINOMA:

Account for about 5-15% of invasive breast carcinoma. Mean age group affected is 50yrs; increase in incidence is mostly attributed to the increase in hormone intake in the form of hormone replacement therapy. They are usually centrally located sometimes can be multicentric and bilateral. Mammogram shows architectural distortion.[10]

Macroscopically they appear as an ill-defined lesion with a diffuse growth pattern. Microscopically the tumor cells are poorly cohesive and appear to be dispersed singly in a fibrous stroma. Few areas show tumor cells that are arranged in cords and infiltrating the stroma. Cells have ovoid nuclei, thin rim of cytoplasm with occasional intracytoplasmic luminal formation.

Other variants of invasive lobular carcinoma includes

(i) Solid pattern – sheets of uniform cells that are tightly packed and shows a lobular arrangement.

(ii) Alveolar pattern – it is similar to that of lobular carcinoma, where the tumor cells are arranged in globular aggregates.

(iii) Pleomorphic lobular pattern- similar to that of typical lobular carcinoma but the tumor cells exhibit marked pleomorphism.

Nodal metastasis is lower than that of IDC, these tumors more commonly metastasize to bone, GIT, uterus, meninges and ovary whereas IDC commonly involves the lungs. Alveolar variant of invasive lobular carcinoma is considered low grade whereas pleomorphic invasive lobular carcinoma has a very poor prognosis.

TUBULAR CARCINOMA:

It is a rare variant of breast carcinoma occurring in the elderly age group which accounts for about 2% of invasive breast cancers[10]. Mammogram shows a characteristic spiculated appearance. Macroscopically the tumor is smaller in size with an average of 1cm. Morphologically there is two types (i) pure type and (ii)the sclerotic type.[22] Pure type has a stellate appearance whereas sclerotic type is more diffuse in nature.

Histopathology:

These tumors are composed of tubules that have a characteristic angulated appearance with open lumens that are lined by a single layer of epithelial cells which are small, regular and exhibits mild nuclear pleomorphism. There should be

minimum 90% of tubules identified in the lesion and should not have multilayering or marked nuclear pleomorphism, if present it excludes the diagnosis of tubular carcinoma. Surrounding stroma shows marked Desmoplastic reaction. Tubular carcinoma can also accompany other epithelial proliferative lesions like ductal carcinoma in situ or lobular neoplasia. This variant has an excellent prognosis as compared to that of invasive ductal carcinoma, NOS.

INVASIVE CRIBRIFORM CARCINOMA:

Common age group affected is around 5th decade of life and accounts for 1-3.5% of breast carcinomas.[10] Mammogram shows speculated appearance with microcalcifications.

Histopathology:

Tumor is predominantly composed of cells arranged in a cribriform pattern and islands which show stromal invasion. Cells exhibit moderate pleomorphism. Tumor islands are surrounded by reactive fibrous stroma. These variants are also found in association with intraductal carcinoma and tubular carcinoma. Invasive cribriform carcinoma has a good prognosis and ten year survival rate is 90%.[10]

MEDULLARY CARCINOMA

Medullary carcinoma accounts for about 1 to 7% of all breast cancers and the common age group affected is between 4th to 5th decade of life.[10] Medullary carcinomas are seen more common in patients with BRCA 1 mutation.[23]

Mammogram shows a well-defined lesion and mimics a benign tumor. Grossly the tumor is well circumscribed, cut surface of which is grey white and homogenous. It has a similar appearance to that of fibroadenoma except that of whorling and slit like spaces. Areas of hemorrhage and necrosis are also noted.

Histopathology:

These tumors have five important characteristic microscopic features:

The tumor should be well circumscribed; tumor cells are predominantly arranged in sheets; there should not be any glandular or tubular structures; surrounding stroma should have dense lympho-plasmacytic infiltrate; tumor cells are round with abundant cytoplasm, Pleomorphic vesicular nuclei and prominent nucleoli, mitosis and atypical giant cells are also seen;. Necrosis and squamous differentiation can also be seen.

Hormonal receptor status shows triple negativity for ER, PR and Her2 neu. Has a better prognosis that infiltrating ductal carcinoma NOS. Only 10% of the cases show lymph node metastasis if the metastatic deposits are found in more than three axillary lymphnodes, it is considered to have a poor prognosis.[10]

MUCIN PRODUCING CARCINOMAS:

It includes all breast carcinomas that have features of producing mucin. There are four important types of mucin producing carcinomas

(i)Mucinous carcinoma:

Accounts for 2% of all breast cancer.[10] Mammogram shows a well-defined, lobulated lesion, looks more or less similar to a benign tumor. Grossly the tumor is soft and cut surface appears gelatinous and glistening. Histopathologically the tumor cells are uniform and are seen floating in lakes of mucin. These mucinous lakes are separated by fibrous tissue into compartments. Few of the tumors have intraepithelial component and rarely exhibits neuroendocrine differentiation. Pure mucinous carcinoma of breast has a very good prognosis, only handful of cases show axillary node metastasis.

(ii)Mucinous cystadenocarcinoma and columnar cell mucinous carcinoma:

These tumors have clinical and radiological features similar to that of infiltrating ductal carcinomas. Macroscopically they are cystic and have a gelatinous appearance. Microscopically both the types comprise of tall columnar mucinous cell with abundant intracytoplasmicmucin and basal nuclei. If they have a cystic appearance then they are called mucinous cystadenocarcinoma or if they have a solid appearance then they are called as columnar cell mucinous carcinoma. These tumor cells can also be arranged in papillary fronds.

(iii)Signet ring cell carcinoma:

There are two variants; one of the types has cytoplasmic lumina which pushes the nuclei to one side of the cell. Other type resembles that of gastric

carcinoma where the cytoplasm is filled with acid mucinous substance and pushes the nuclei towards one pole.

NEUROENDOCRINE TUMORS:

It accounts for about 2-5% of breast cancers. It occurs between 6th to 7th decades of life.[24] Mammogram shows a well circumscribed lesion. Macroscopically they appear as an expansile tumor which can be gelatinous at times. Microscopy shows tumor cells arranged in characteristic nesting pattern and in solid sheets with peripheral palisading of cells. There are different subtypes which includes

(i)Solid neuroendocrine carcinoma

Tumor cells are arranged in nests and trabeculae separated by fibrovascularstroma. They exhibit a lobular pattern. Cells are small with round to oval nuclei and stippled chromatin. Mitotic figures are also noted

(ii)Small cell / oat cell carcinoma:

Tumor shows an invasive growth pattern and composed of cells that are small with hyperchromatic nuclei. They appear similar to that of small cell carcinoma occurring in the lungs both morphologically and immunohistochemically.

(iii)Large cell neuroendocrine carcinoma:

Cells are arranged in clusters, they are large with abundant cytoplasm, vesicular nuclei and numerous mitotic figures are also noted

These neuroendocrine tumors can be graded as well, moderately and poorly differentiated. Well differentiated carcinomas have a better prognosis than high grade tumor.

INVASIVE PAPILLARY CARCINOMA:

It accounts for about 1-2% of invasive breast carcinomas.[10] These tumors are diagnosed in women in the post-menopausal age group. Mammogram shows nodular densities that are multiple and at times can be lobulated. Macroscopically tumor appears fairly circumscribed and has features similar to that of invasive ductal carcinoma nos.

Histopathology:

Tumor is well circumscribed and the tumor cells are arranged in a papillary pattern. Cells have amphophilic cytoplasm, exhibiting moderate amount of pleomorphism and few may show apocrine changes. Surrounding stroma is scant and these tumors can also show abundant extracellular mucin. More than 75% of cases are associated with DCIS component. It has a better prognosis compared to that of invasive ductal carcinoma, NOS.[10]

INVASIVE MICROPAPILLARY CARCINOMA:

This variant accounts for less than 2% of all breast cancer cases. They usually present themselves at first clinically with axillary lymph node metastasis.[25] Macroscopically they have a lobulated outline indicating they have an invasive growth. Microscopy shows clusters of tumor cells exhibiting moderate nuclear pleomorphism and they are surrounded by a clear stromal space which has been created artifactually.

APOCRINE CARCINOMA:

It constitutes about 4% of all invasive carcinomas. Mammogram shows feature similar to that of invasive ductal carcinoma, NOS. Microscopically any type of invasive breast carcinoma can show apocrine changes. The term apocrine carcinoma is only applicable when there are more than 90% of tumor cells showing apocrine changes. These changes are also noted in ductal / lobular carcinomas in situ. There are two types of cells namely type A and type B.[10] Type A cell are large with abundant eosinophilic cytoplasm, has globoid nuclei with prominent nucleoli. They appear like granular cell tumors. Type B cells have abundant cytoplasm with many vacuolations within. These cells are similar to that of macrophage.

METAPLASTIC CARCINOMA:

It accounts for less than 1% of breast carcinoma. They are heterogenous tumors which can be

Epithelial:

(i) Squamous

(ii) Adenocarcinoma with spindle cell differentiation

(iii) Adenosquamous, including mucoepidermoid (or)

Mixed epithelial-mesenchymal:

(i)Carcinoma with chondroid metaplasia

(ii)Carcinoma with osseous metaplasia

(iii)Carcinosarcoma

*Epithelial tumors - Squamous cell carcinoma: The carcinoma is composed of metaplastic squamous cells. Can be of keratinizing type/non keratinizing type or spindle in type. They are not derived from skin or metastasis from elsewhere.

*Adenocarcinoma with spindle cell metaplasia: The tumor is well circumscribed composed of tubules of adenocarcinoma interspersed with atypical spindle cells.

*Adenosquamous carcinoma: These tumors composed of characteristic adenocarcinomatous component interspersed with solid nests of squamous differentiation

*Mixed epithelial/mesenchymal metaplastic carcinomas:

They are considered as matrix producing carcinomas. Adenocarcinoma is mixed with areas showing mesenchymal components like chondroid or osseous differentiation. Sometimes it can be full blown sarcoma. Usually the mesenchymal component appears benign. If they are malignant then they are termed as carcinosarcoma.

Generally metaplastic tumors of pure epithelial type and mixed epithelial/mesenchymal type has a five year survival rate of about 60%. Among which carcinosarcomas are considered as a highly aggressive variant.

LIPID RICH CARCINOMA:

It accounts for about 1-6% of breast carcinoma cases. Patients often present with Paget's disease of nipple. Histopathologically these tumors show grade III morphology, the cells are large with foamy cytoplasm. There should be a minimum of 90% of neoplastic cells containing neutral lipids that should be demonstrable.

SECRETORY CARCINOMA:

It is a rare tumor with frequency of 0.15% of all breast carcinomas. It affects children as well as adults and is situated near the areola. Macroscopically they appear as a well circumscribed greyish white to tan nodule. Histopathologically they are found in three patterns

(i) Microcystic pattern, (ii) solid pattern and (iii) a tubular pattern.

Cells are large with abundant pale granular cytoplasm, round to oval nuclei with occasional small nucleoli. Intracytoplasmic lumina are seen. Sometimes these lumina coalesce together and form microcystic structure. Secretions are also found within the lumina which is strongly PAS positive. Has a very good prognosis for children but has an aggressive behavior in adults.

ONCOCYTIC CARCINOMA:

Incidence is very rare. They can be misdiagnosed as apocrine carcinoma. Seen in patients around 60yrs of age. Histopathologically the tumor is circumscribed and composed of cells arranged in a glandular pattern and solid sheets. The cells have abundant eosinophilic granular cytoplasm, round to oval monomorphic nuclei with inconspicuous nucleoli. More than 70% of cells should have oncocytic appearance.[10]

ADENOID CYSTIC CARCINOMA:

Its a rare type of breast carcinomas. It has low aggressive behaviour and is a counterpart of histologically similar tumor arising in salivary glands. Usually these tumors are bilateral and are situated around sub-periareolar region. Macroscopically they are well circumscribed lesion, cut surface of which appears pink or tan. Histopathologically three patterns can be made out (i) trabecular-tubular (ii) cribriform (iii) solid. Classically the cells are arranged in fenestrated nests or in tubules, cells can be either basaloid in nature with scant cytoplasm or cells with eosinophilic abundant cytoplasm with basaloid type of nucleus. It is a low grade malignant tumor and has a good prognosis, doesn't spread via lymphatics.

ACINIC CELL CARCINOMA:

A very rare tumor is observed in females around 5th decade of life. These tumors are considered as the counterpart of similar tumor that is found in the parotid gland. Microscopy shows cells are arranged in microcystic and microglandular pattern, with abundant eosinophilic granular cytoplasm, pleomorphic nuclei and occasional prominent nucleoli. Mitotic figures can be upto 15mitoses/10Hpf.[10]

GLYCOGEN RICH, CLEAR CELL CARCINOMA:

It accounts for about 1-3% of all invasive breast carcinomas. Median age is 57 yrs. Macroscopically it appears similar to that of invasive or DCIS. Histopathologically the cells are arranged in solid nests and few of them show the tubular or papillary pattern, the cells are polygonal in shape, shows clear cytoplasm with hyperchromatic nuclei and prominent nucleoli. They are also associated with ductal carcinoma in situ component. More than 90% of cells should have clear cytoplasm containing glycogen. These tumors have more aggressive behaviour than usual ductal carcinoma. Most of the cases show axillary lymph node metastasis.

SEBACEOUS CARCINOMA:

It's a very rare variant. Gross appearance shows a well delineated tumor, cut surface of which appears yellow. Histopathologically tumor cells are arranged in lobules composed of sebaceous cells mixed with small ovoid to spindle cells. The cytoplasm of sebaceous cells is abundant with vacuolations whereas the cytoplasm of the spindle/ ovoid cells is scant. Nuclei of both the cells appear vesicular with occasional prominent nucleoli. Few mitotic figures are noted.

INFLAMMATORY CARCINOMA:

These tumors show a characteristic feature of the dermal lymphatic invasion from an underlying invasive ductal carcinoma. Most commonly found in

women of a younger age group. Grossly there is edema, peau d'orange and induarated appearance of skin overlying the breast. Puckering of nipple can also be noted. Microscopy shows tumor cells with grade 3 morphology with evidence of dermal lymphatic invasion. Despite the name these tumors show only minimal lympho-plasmacytic infiltrate. These tumors are straight away graded as T4d and have a very poor prognosis with 5 year survival rates is less than 5%.[10]

NOTTINGHAM MODIFICATION OF SCARFF – BLOOM – RICHARDSON GRADING:[26]

This grading system was proposed by Ellis and Elston. It is a semi quantitative test

TUBULE FORMATION:

1 Point	>75%
2 Points	10-75%
3 Points	< 10%

NUCLEAR PLEOMORPHISM:

1 Point	Minimal pleomorphism
2 Points	Moderate pleomorphism
3 Points	marked pleomorphism

MITOTIC COUNT:

Points are provided depending upon the number of mitosis counted in a particular determined field area

Field diameter (mm)	0.44	0.59	0.63
Filed area (mm2)	0.152	0.274	0.312
Mitotic count			
1 point	0-5	0-9	0-11
2 points	6-10	10-19	12-22
3 points	>11	>20	>23

GRADES:

Grade 1: well differentiated breast carcinoma : 3-5 points

Grade 2: moderately differentiated breast carcinoma : 6-7 points

Grade 3: poorly differentiated breast carcinoma : 8-9 points

TNM CLASSIFICATION OF CARCINOMAS OF THE BREAST:[27]

T: Primary Tumor

TX: Primary tumor cannot be assessed

T0: No evidence of primary tumor

Tis: Carcinoma in situ

Tis (DCIS): Ductal carcinoma in situ

Tis (LCIS): Lobular carcinoma in situ

Tis (Paget): Paget disease of the nipple with no invasive or in-situ carcinomatous component

If Paget disease is found in association with invasive or carcinoma in situ component, then they are classified according to the size of the tumor.

T 1: Tumor = 2 cm in greatest dimension

T 1 mi: Microinvasion = 0.1 cm in greatest dimension

T 1a Tumor > 0.1 cm but = 0. 5 cm in greatest dimension

T 1b Tumor > 0.5 cm but = 1 cm in greatest dimension

T 1c Tumor > 1 cm but = 2 cm in greatest dimension

T 2 Tumor > 2 cm but = 5 cm in greatest dimension

T 3 Tumor > 5 cm in greatest dimension

T4 Tumor of any size with direct extension to chest wall or skin

T4a to T4d Note:Chest wall includes ribs, intercostal muscles, and serratus anterior muscle but not pectoral muscle.

T4a Extension to chest wall

T4b Edema (including peau d'orange), or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast

T4c Both 4a and 4b, above

T4d Inflammatory carcinoma

STAGING OF NODAL METASTASIS:

N – Regional Lymph Nodes

NX: Regional lymph nodes cannot be assessed (e.g. previously removed)

N0: No regional lymph node metastasis

N1: Metastasis in movable ipsilateral axillary lymph node(s)

N2a: Metastasis to ipsilateral axillary lymph node(s) fixed to one another or to other structures

N2b: Metastasis only in to ipsilateral internal mammary lymph node(s) and in the absence of clinically evident axillary lymph node metastasis

N3a: Metastasis to ipsilateral infraclavicular lymph node(s)

N3b: Metastasis to ipsilateral internal mammary and axillary lymph nodes

N3c: Metastasis to ipsilateral supraclavicular lymph node(s)

STAGING OF METASTASIS:

M: Distant Metastasis

MX: Distant metastasis cannot be assessed

M0: No distant metastasis

M1: Distant metastasis

pTNM Pathological Classification

pT: Primary Tumor

pN: Regional Lymph Nodes

pNX: Regional lymph nodes cannot be assessed which are previously removed

pN0: No regional lymph node metastasis

pN0(i-): with negative immunohistochemsitry

pN0(i+): tumor cells in regional lymphnodes less than 0.2 cm (detected by H and E or IHC)

pN0(mol-): negative molecular findings using RT-PCR

pN0(mol+): positive molecular findings on using RT-PCR, but without histological or immunohistochemical findings.

pN1a: Metastasis in 1-3 axillary lymph node(s), including at least one larger than 2 mm in greatest dimension

pN1b: Internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN1c: Metastasis in 1 - 3 axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN2a: Metastasis in 4-9 axillary lymph nodes, including at least one that is larger than 2 mm

pN2b: Metastasis in clinically apparent internal mammary lymph node(s), in the absence of axillary lymph node metastasis

pN3a: Metastasis in 10 or more axillary lymph nodes (at least one larger than 2 mm) or metastasis in infraclavicular lymph nodes

pN3b Metastasis in clinically apparent internal mammary lymph node(s) in the presence of one or more positive axillary lymph node(s); or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with sentinel node biopsy but not clinically seen.

pN3c Metastasis in the ipsilateral supraclavicular lymph node(s)

DISTANT METASTASIS:

M0: no evidence of distant metastasis (clinically and radiologically)

Cm0(i+): no radiological/clinical evidence of metastasi, but molecular or microscopic detection of tumor deposits in circulating blood, bone marrow, non-regional nodal tissue that are =0.2 cm

M1: distant detectable metastasis determined clinically or radiographically and/or histological evidence of deposits >0.2cm

TNM STAGING OF BREAST CARCINOMA[29]

Stage 0	Tis	N0	M0
Stage 1	T1	N0	M0
Stage II A	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage II B	T2	N1	M0
	T3	N0	M0
Stage III A	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1,N2	M0
Stage III B	T4	N0,N1,N2	M0
Stage III C	Any T	N3	M0
Stage IV	Any T	Any N	M1

PROGNOSTIC AND PREDICTIVE FACTORS:

Prognosis of breast carcinoma depends mainly upon clinical and pathological factors. Most of the women diagnosed are expected to have a normal life expectancy but rest of the patients have a poor prognosis, which includes those who presented with distant metastasis or inflammatory carcinoma.

Prognosis of breast cancer depends upon two entities:[1]

(i) Extent of the cancer and

(ii) Biology of the cancer.

It is essential to find out the prognosis of the patient which can be helpful for counselling them and also devising a treatment strategy accordingly. We can also motivate and plan for clinical trials.

PROGNOSTIC FACTORS DEPENDING UPON THE EXTENT OF TUMOR ARE:

1. Invasive carcinoma vs ductal carcinoma in-situ:

The presence or absence of invasiveness is considered as one of the major prognostic factor. Ductal carcinoma in situ in general has an excellent prognosis. Rarely they are invasive and mastectomy can provide surgical cure. But if DCIS component is found to be multicentric, then there is a high possibility of finding an invasive occult carcinoma elsewhere in the breast.[27] Comedocarcinoma variant of

DCIS, is a high grade lesion that can metastasize to lymph node without showing any features of invasion.

2. Lymph node metastasis:

Axillary lymph node metastasis is one of the most important prognostic factor among all the other factors. It should always be evaluated by histopathological examination of a biopsied specimen because clinical examination for nodal involvement can give either a false positive results, were reactive nodes become palpable or false negative results, were micrometastasis cannot be palpated. If the axillary nodes are free of tumor then the 10 year survival rate is about 70% - 80%, when one to three nodes are positive the survival rate is reduced by 35% to 40%. If ten and more nodes are involved the survival rate is only 10% to 15%.[1] In breast carcinoma all the lymphatic channels initially drains into the first lymph node and it is known as sentinel lymph node. This particular node can be identified using dyes and biopsy is taken from them to rule out metastasis, if sentinel lymph node shows no evidence of metastasis then rest of the nodes are highly unlikely to show any evidence of metastasis. Few of the patients' presents with recurrence of tumour and distant metastasis even in the absence of axillary lymph node involvement, due to presence of metastasis in internal mammary lymph nodes or rarely by a haematogenic spread.

3. Distant metastasis:

If the patients are diagnosed to have distant metastasis, then there is no cure available. Distant metastasis if present is considered as an important prognostic factor than that of axillary lymph node metastasis.

4. Size of the tumor:

When the tumor size increases the prognosis becomes poor. The chance of nodal metastasis is directly proportional to the tumor size. If the size of a tumor is 1cm and there is no nodal metastasis, the 10 year survival is around 90%. If size is 2cm then survival rate comes down to 77%. When both ER and Her 2 receptor status are negative, the prognosis is regarded as poor even if the tumor is less than 1cm without any nodal involvement. Microscopic evaluation of tumor size has a greater significance than that of measuring the size macroscopically. The term minimal breast carcinoma is used when all the carcinoma in-situ components and invasive component are less than or equal to 1cm.[1]

5. Local extension of the lesion:

When breast carcinoma involves the overlying skin or underlying skeletal muscle, the surgical treatment becomes difficult. But recently due to early detection of breast cancer and better awareness, this has become a rare presentation.

6. Inflammatory carcinoma:

Breast cancers can also present with thickened and erythematous skin that becomes oedematous and adheres with Coopers ligament. Grossly it appears as surface of orange peel hence they are also called as peau d'orange in appearance. These tumors are not palpable and present only with the symptom of swollen breast which can be mistaken for a breast abscess. Microscopically the characteristic infiltration of dermal lymphatic channels by tumor cells are seen which completely blocks the lymphatic drainage. As they don't have any characteristic histological or molecular evidence they are termed as inflammatory carcinoma, has a very grave prognosis and 3 year survival rates is only around 3% to 10%.[1]

7. Lymphovascular invasion:

More than half of invasive breast carcinoma cases show features of tumor cells infiltrating into the lymphatic spaces and blood vessels. It indicates that there is a high chance of recurrence even though there may not be any nodal metastasis and usually correlates with the presence of axillary lymph node metastasis. It is a poor prognostic factor.[28]

PROGNOSTIC FACTORS RELATED TO TUMOR BIOLOGY:

1. Molecular subtype:

ER, PR and Her 2 receptor expression by breast tumors play an important role in determining the prognosis of the patient. ER positive tumors have a better prognosis in terms of longer disease free survival rate than others.

2. Cytoarchitectural types:

Morphological variants of invasive breast carcinoma in general have a better prognosis than invasive breast carcinoma not otherwise specified. Tubular, lobular, papillary and adenoid cystic carcinoma variants generally have a good prognosis. Whereas metaplastic carcinoma, micropapillary carcinoma and signet ring cell variant of lobular carcinoma has a very poor prognosis.

3. Histological grade:

Invasive breast carcinomas are graded using a Nottingham modification of the Bloom-Richardson system. The system includes parameters like tubule formation, nuclear atypia and number of mitosis detected. So depending upon semi-qualitative assessment of the parameters, a final grading of well, moderate and poor differentiation is made out.[26]

4. Proliferative index:

Proliferation of tumor cells can be assessed by mitotic count measurement in a particular field area or by using immunohistochemical markers like MIB-1 or Ki-67. Proliferative index is important for ER positive and HER 2 negative cases whereas ER negative and HER 2 positive receptor cases always has a high proliferative index.[1] When there is increased proliferation it denotes a poor prognosis. But these patients can respond better to chemotherapy than other modalities of treatment.

5. ER, PR and HER2 receptors:

Breast carcinoma cases that express both ER and PR receptors respond well to hormonal treatment and hence have a good prognosis. Around 80% of patients with both the receptors positive respond well to treatment. But if there is expression of only ER or PR receptor alone the response to hormonal treatment is reduced to about 40%. If the patient is ER negative then the patient may respond well to chemotherapy than hormonal therapy, HER2 expression denotes the patients have a poor survival rate.

Other factors that play a substantial role in prognosis are:

Age at presentation:

Breast carcinoma detected below the age group of 50 years has a better prognosis than those who were detected above 50 years. Whereas younger women detected with breast cancer who were below the age group of 35 years has prognosis similar to that seen in older women. These patients will have a high grade lesion and chances of relapse are significantly higher.[29]

BRCA 1:

Breast carcinoma with BRCA 1 mutation has an overall shorter disease free interval. BRCA 1 expression on tumor cells can be detected using immunohistochemical stains. Depending upon the expression prognosis varies. If there is a reduced cytoplasmic expression the tumor has more chances of relapsing, when nuclear expression is reduced the tumor tends to have a poor prognosis.

Pregnancy:

When breast carcinoma manifests at the time of pregnancy or during lactation, it is found that the tumor shows low expression of ER/PR receptors and high expression of HER 2 receptors which in turn indicates the prognosis is poor and 5 year survival rate is only around 15% to 35%.[30]

Margins of tumor:

Tumors with infiltrative margins have a poor prognosis than those with pushing type of margins. It is usually noted in medullary type of carcinomas.

Tumor necrosis:

Necrosis is usually noted in high grade lesions and indicates there are more chances of nodal metastasis which points towards a poor prognosis.

Stromal reaction:

Breast carcinomas with no inflammatory reaction have a better prognosis and reduced incidence of nodal metastasis. Tumors exhibiting a greater degree of elastosis respond well to hormonal management than those with no elastosis. Also if there is a central fibrotic scar in the portion of a tumor it indicates hypoxia and lymph angiogenesis which are considered as an indicator for a bad prognosis.

An American joint committee on cancer has proposed a classification that correlates the disease with ten year survival rates.

American joint committee on cancer and Union Contre Le cancer staging:[31]

STAGE	PRIMARY CANCER	LYMPHNODES (metastasis)	DISTANT METASTASIS	10-YEAR SURVIVAL RATE
0	DCIS or LCIS	No	Absent	92%
I	Invasive ca ≤ 2 cm	No / micrometastasis	Absent	87%
II	Invasive ca > 2cm Invasive ca > 5cm but ≤ 5cm	1 to 3 +ve 0 to 3 +ve	Absent Absent	65%
III	Invasive ca > 5cm Any size invasive ca Invasive ca with skin or chest wall involvement or inflammatory ca	-ve or +ve ≥ 4 +ve -ve or +ve	Absent Absent Absent	40%
IV	Any size invasive carcinoma	-ve or +ve	present	5%

IMMUNOHISTOCHEMISTRY:

Immunohistochemistry is defined as the process of detecting antigens (e.g., proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.

So with the help of immunohistochemistry in breast carcinoma, prognosis and predictive value for the patients can be determined. But recently it has been identified that the expression of receptors using IHC markers is helpful in prediction of response to hormonal therapy than that of prognosis.

ESTROGEN RECEPTOR:

Estrogen receptor positive breast carcinomas are considered to have a good prognosis because they respond well to hormonal treatment. Recent studies indicate more than 70% of breast cancer cases in general shows ER receptor positivity, one of the study conducted portrays that nearly all patients with grade I tumor are found to be ER positive.[32] Many studies stress the fact that PR receptor positive cases can also determine the outcome of patients, it is derived from the stats that depicts patients with ER and PR positive status together has a better prognosis than that compared to ER positive and PR negative receptors.

As with any IHC studies there are some factors which play an important role in the outcome of the result. These include fixation time, processing quality,

antigen retrieval method and a clone of the antibody used which have been described below.

Specimen type and fixation time:

IHC studies in breast carcinoma can be done either in a biopsy sample or in a resected section. Few studies showed that IHC results were false negative in about 9% in resected sections than in biopsied specimen. The sole reason attributed to a false negative result is due to inadequate fixation of the sample in formalin. So it was proposed that the least required time to fix resected tissue in formalin is 6 to 8 hours for better estimation of ER positivity.[33]

Selection of type of antibody:

Choice of antibody used in IHC study is of prime importance because it should be strong enough to attach to the corresponding antigens and the results obtained should correlate with the clinical outcome of the patient. 1D5, 6F11 and SP1 are the three strong antibodies that provide better results.[34] Out of which SP1 monoclonal antibody is considered superior.

Interpretation of the IHC expression depends upon two factors

(i) Distribution of staining among tumor cells

(ii) Intensity of staining

Though using IHC for determining ER receptor status has been used for a long time since, still there were some errors among pathologists in classifying weak ER positive cases and ER negative cases. Most of the weak ER positive cases are mislabeled as ER negative cases, thus devoiding them of treatment with hormones. So to prevent the discordance, a nine points scoring system was proposed by Allerd, Harvey et al and it was named as Allred scoring system. It is a semiquantitative test which takes into account the proportion of cells stained and the intensity of staining.[35]

Proportion score	Nuclear staining	Intensity score	Observation (intensity)
0	No staining	0	No staining
1	< 1%	1	Weak
2	1-9%	2	Intermediate
3	10-32%	3	strong
4	33-65%		
5	66-100%		

The final Allred score is the sum of proportion and intensity scores, if the total score is 0-2 then the expression is negative, when the score is 3-8 receptor expression is considered positive.

Estimation of concentration of the receptors within the tumor is of clinical importance as they influence the optimal selection of treatment for the patients by clinicians and in turn reflects the prognosis and survival of the patient. So by using the Allred score, a cut off value for the treatment of breast carcinoma is formulated by Robin Leake and et al which is as follows:[36]

score	Response to treatment
0	Endocrine treatment will not work
2-3	20% chance of response to endocrine treatment
4-6	50% chance of response to endocrine treatment
7-8	75% chance of response to endocrine treatment

HUMAN EPIDERMAL RECEPTOR PROTEIN – 2:

It is an oncogene protein and one of the transmembrane receptor glycoprotein belonging to the epidermal growth receptor family. In breast carcinomas expression of HER 2 receptors play a significant role in prognosis and outcome of the patient. HER 2 receptor positivity predicts the sensitivity of treatment to anthracycline based drug regimens and can be treated with

trastuzumab which is a form of targeted therapy, whereas they don't respond well to hormonal treatment. There are also factors similar to that of ER receptor which affects the outcome of the result. Common methods for determining the HER 2 receptor status are by IHC or FISH. But there is a difference in concordance values between the two. According to the new guidelines proposed by ASCO-CAP, it is necessary that the sections should be fixed for a minimum of 6 hrs to a maximum of 48 hrs, core biopsy specimen should be fixed minimum for one hour. They have also created FDA scoring system to reduce the concordance between FISH and IHC results.

FDA scoring system for HER 2:[37]

Score	Observation
0	No immunostaining
1+	Weak immunostaining, < 30% of tumor cells
2+	Complete membranous staining, either uniform or weak in at least 10% of cells
3+	Uniform intense membranous staining in at least 30% of cells

BRCA 1 :

BRCA 1 located on chromosome 17 is found to be mutated in many familial breast and ovarian carcinomas. It is a type of germline mutation showing loss of hetrozygosity pattern suggesting to the fact that these are tumor suppressor gene. BRCA 1 has A growth regulatory function of breast epithelial cells. So when there is a BRCA 1 mutation it acts as a negative regulation of proliferation of epithelial cells. BRCA 1 and BRCA 2 accounts for about 80-90% of all the familial / hereditary cancers. BRCA 1 gene is of prime importance is that these susceptible genes when undergo mutational changes and being inherited by an individual not only causes risk of getting breast carcinoma but also prone to develop ovarian carcinoma around 10-20%.[6] Whereas the propensity to cause breast cancer is far more less that of breast cancer caused by mutated BRCA 1 gene. These two genes are also likely to cause epithelial cancers mostly involving the prostate and pancreas. BRCA1 plays an important role in check point activation and DNA repair, thereby preventing DNA damage. BRCA 2 has a significant role in homologous recombination. When BRCA 1 gene is mutated, risk of acquiring breast carcinoma is 56% to 90% whereas when BRCA 2 is mutated the risk is 10%.[6] Patients with carcinoma breast showing BRCA 1 mutation can also have a family history of ovarian carcinoma.

Usually carcinoma breast with BRCA 1 mutations tend to be higher grade (grade 3) and are poorly differentiated. These carcinomas also do not express

estrogen receptor. BRCA 1 can also undergo sporadic type of mutation, mostly due to hypermethylation of promoter region.

By using immunohistochemical studies based on the principle of directing antibody to the target site specific antigen, BRCA 1 expression can be studied in tumor cells. Normally breast tumor cells express BRCA 1 which imparts a brown colour when examined microscopically. Hence depending upon the intensity of staining in the nucleus or cytoplasm, a scoring system is devised:[38]

score	**Staining of cells (% of positive cells)**
0	No staining
1+	< 10% positive cells
2+	10-25% positive cells
3+	25-50% positive cells
4+	50-70% positive cells
5+	> 75% positive cells

So 0 is considered as complete loss of BRCA 1 expression, 2 is mild expression, 3 is moderate expression, 4 and 5 are normal expression.[38]

Clinical significance of detecting the mutation is that the chances of developing carcinoma in both the breast are significantly high. Women who were detected positive for BRCA 1 mutation without any evidence of breast carcinoma can be benefited by prophylactic bilateral mastectomy were the relative risk reduction is around 90-100%.[39] Bilateral oophorectomy can also reduce the risk of acquiring breast carcinoma by 47-68%.[40] In a study around 49% of cases with BRCA 1 mutation who underwent breast conservation surgery developed recurrence of carcinoma and 42% developed carcinoma in the contralateral breast.[41] The risk of acquiring carcinoma in contralateral breast in patients with BRCA 1 muattion, after 10 years of follow up were 43.4%. few of the studies showed that usage of tamoxifen in the adjuvant therapy as a part of treatment can reduce the incidence of carcinoma recurrence after breast conservation surgery and also reduces the risk of acquiring carcinoma in the contralateral breast and there is a 50% reduction of contralateral carcinoma in patients who received tamoxifen as an part of adjuvant chemotherapy.[6]

MATERIALS AND METHODS:

Study design and selection of study population:

This is a retrospective study. Cases reported as carcinoma breast were retrieved from the archives of department of pathology, between the years 2012 to 2015. Breast carcinomas reported in trucut biopsy and lumpectomy specimens were the exclusion criteria.

Details of age of the patient, stage of the disease, radio diagnosis were obtained from the requisition form sent by clinicians and the details of pathological aspects like gross findings etc were retrieved from the archives of pathology.

For histopathological examination of the tissue with breast carcinoma, paraffin blocks of the suitable representative section were retrieved from the department of pathology and they are cut to a thickness of 4 microns with the help of semi-automated microtome. The sections are then stained with Haematoxylin and Eosin manually. These slides were used for assessing the grade of the disease. The Haematoxylin and Eosin stained slides were examined for adequacy and as a rule of thumb we took a minimum of 60-70% well-preserved tumor area and tissues with less than 10% of necrotic area for IHC. This was done to prevent staining artefacts and for obtaining better results. Corresponding blocks were retrieved and four thin (4 to 5 microns) sections were taken to perform immunohistochemical study on ER, PR, HER2 and BRCA 1.

HAEMATOXYLIN AND EOSIN STAINING:

Two important compositions of the stains are:

1. Harris Haematoxylin
2. Eosin Y

PROCEDURE:

With the help of microtome, 4-5 micron thin sections were floated on slides that are coated with egg albumin. The following staining procedure is carried as followed:

1. As a first step for deparaffinization, xylene is used
2. Section is then hydrated using graded alcohols to remove the xylene
3. Now wash the section with water so that excess alcohol is removed
4. Slides are then covered with Harris Haematoxylin for 10 minutes
5. Slides are washed in running tap water for 5 minutes to remove the Haematoxylin, we can observe that the section gradually turn to blue and is called bluing.
6. 1% acid alcohol (1% hydrochloric acid in 70% alcohol) is used to flood slides for 10 seconds. This is called the step of differentiation. As it removes the excess stain, it is also known as regressive staining.
7. Tap water is used to wash the slides for 10 minutes
8. Slides are then stained with eosin Y for 10 to 15 minutes

9. Stained slide is washed with running tap water for 3 minutes
10. Now the sections are dehydrated using graded concentration of alcohol
11. Xylene is the used as a clearing agent
12. Finally DPX (distyrene phthalate xylene) is used for mounting.

IMMUNOHISTOCHEMICAL STAINING:

TEST PRINCIPLE:

It is a two stage process. The first step involves the binding of the primary antibody to the targeted antigen in tissue tested. The second step is to identify the primary antibody bound to the antigen using a secondary antibody by a colorimetric reaction. The secondary antibody is bound to a dextran polymer with the help of horseradish peroxidase enzyme and an attached chromogen which is responsible for the production of characteristic colour.

Immunohistochemical reagents used are:

Antibody reagent	Clone
ER	Clone EP1 by DAKO
PR	Clone PgR 636 by DAKO
HER2	Anti-c-erbB-2 Clone CB11 by biogenex
BRCA1	Anti-BRCA1 Protein [Polyclonal][Rabbit]

Retrieval of the antigen:

When the tissue is fixed by formalin the antigenic site gets obscured. The masking effect is due to the formation of cross linkage. For the antibody to bind to the specific antigenic site, the antigen has to be exposed which are covered by the cross linkage. The method by which the antigenic site is made to expose so that the antibodies can bind freely is called as antigen retrieval.

Few of the methods for antigen retrieval are:[42]

1. Pressure cooker method
2. Proteolytic digestion method
3. Microwave method

We have used the pressure cooker method for this study. It is based on the principle that the antigenic site can be exposed by the action of pressure and heat in concordance. Antigen retrieval is an important step, after dewaxing and hydration of the section by IHC staining procedure. Antigen retrieval by pressure cooker is done for 10mins in EDTA buffer at an alkaline pH of 9.

Reagents used are:

1. Ethylene Diamine Tetra Acetic Acid buffer at pH 9.

2. 3% Hydrogen Peroxide in distilled water – Is used to prevent endogenous peroxidase action, thereby preventing nonspecific background staining.
3. A solution of 0.01 M Phosphate Buffered Saline (PBS) is prepared with a pH of 7.6. They are prepared by adding the following substance in distilled water.

    Dibasic Sodium Phosphate, Anhydrate 17.5g

    Monobasic Potassium Phosphate, Anhydrate 2.5g

    Sodium chloride 17 g

4. Blocking: The reagent used is casein in buffered saline with 15 milli mole Sodium Azide, which blocks non-specific protein binding
5. Primary antibody against BRCA 1 from Biogenex
6. Poly HRP reagent – Horse Radish Peroxidase Enzyme
7. DAB (3,3'DiaminoBenzdine Tetra Hydrochloride) is used the chromogen. This causes permanent brown precipitate.
8. Harris Haematoxylin
9. Distrene Dibutyl Phthalate Xylene - for mounting

PROCEDURE:

With the help of microtome, 4-5 micron thin sections were floated on slides that are coated with Poly L Lysine in a water bath maintained at 37 degree Celcius.[43] The following staining procedure is carried as followed:

1. Deparaffiniztion of slides by xylene
2. Rehydration by graded alcohol which removes xylene
3. Washed in running tap water for 1 minute
4. Antigen retrieval by pressure cooker method in citrate buffer at pH 6 or EDTA buffer with pH 9 for 10 minutes
5. Wash with running tap water
6. Wash with TBS /PBS buffer at least for 5 minutes
7. The slides are then immersed in 0.3% hydrogen peroxide for 20 minutes to block the non-specific background staining caused by endogenous peroxidase enzyme
8. Slides are washed with PBS buffer for 5 mins
9. Non-specific reaction of the reagent antibody with other tissue antigens is prevented by using a power block containing Casein and Sodium Azide.
10. Slides are now incubated with BRCA 1 primary antibody for one hour
11. Slides are washed with PBS buffer three times for 3 mins
12. The sections are covered with super sensitive polymer HRP for 30 minutes
13. Wash the slide thrice in PBS

14. Chromogen DAB is added and incubated for 5 to 8 minutes, were the colour of the section turns brown.
15. Wash with PBS buffer three times for 5 mins and then in tap water
16. Counter stain with Harris Hematoxylin for1 min
17. Slides are then washed with tap water
18. Sections are cleared with xylene and mounted with DPX.

Blocks were also cut and stained with immunohistochemical markers for ER, PR and HER2, with positive controls for all three cases.

The stained slides are examined for the expression of BRCA 1 and compared with the controls mentioned earlier. The intensity of the staining is scored arbitrarily as there are no established scoring systems in consideration.

DATA ANALYSIS:

Parameters that are entered in master chart are:

Age of the patient

Histological type of tumor

Size of tumor

Grade

TNM staging

Nodal involvement

Staining properties by ER, PR, HER 2 and BRCA 1.

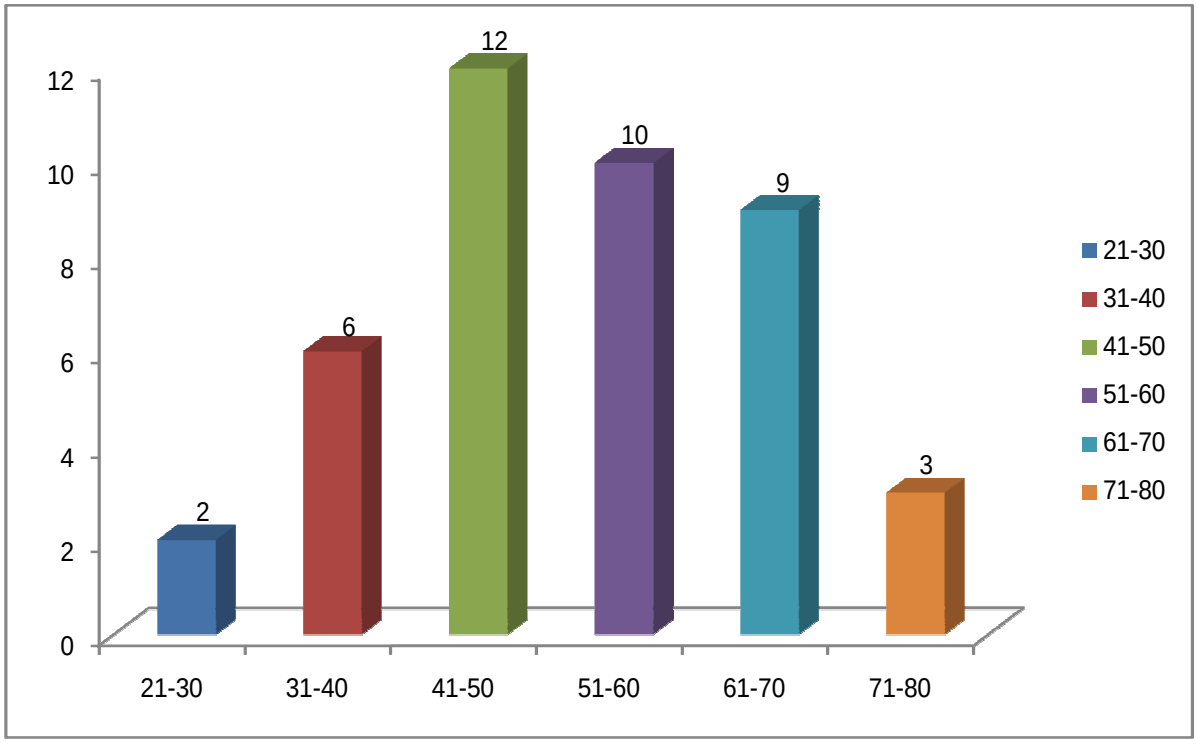
The information was entered into a Microsoft excel worksheet.

RESULTS AND OBSERVATIONS

The department of pathology of this institute received 18,885 biopsy specimens over a period of January 2012 to April 2015 of which 3064 were reported as malignant lesions. Of these 3064 malignancies 97 were breast carcinomas with an incidence of 3.1%. Out of 97 cases 42 were selected for our study using the inclusion and exclusion criteria mentioned before.

CHART 1:

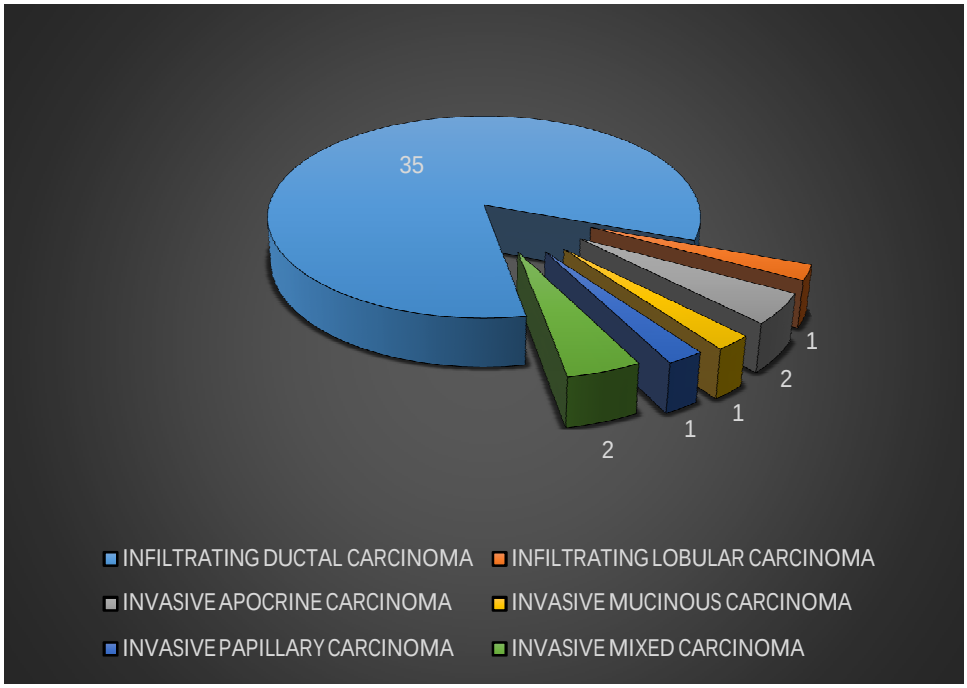
AGE DISTRIBUTION



The above chart shows age distribution of breast carcinoma cases taken up for study, out of which more than half the cases are distributed between the age group of 40-60 years.

CHART 2

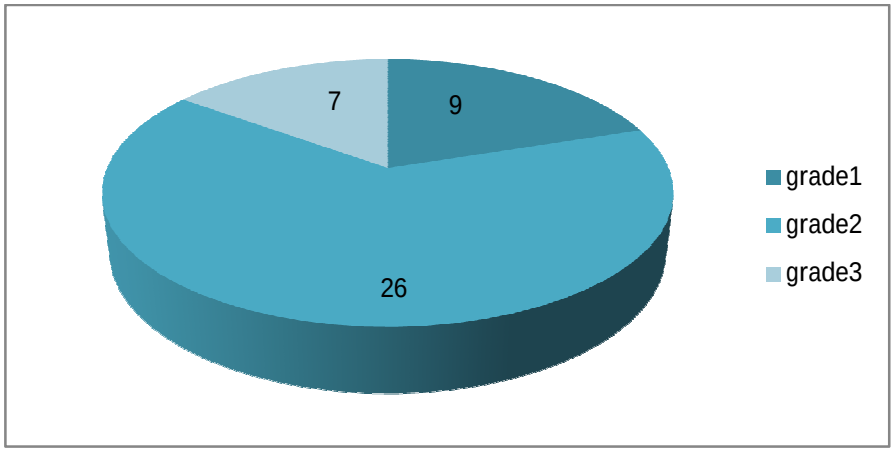
HISTOLOGICAL VARIANTS OF BREAST CARCINOMA:



The above chart shows the distribution of various histological types taken in our study and majority of the cases are found to be invasive ductal carcinoma, not otherwise specified.

CHART 3

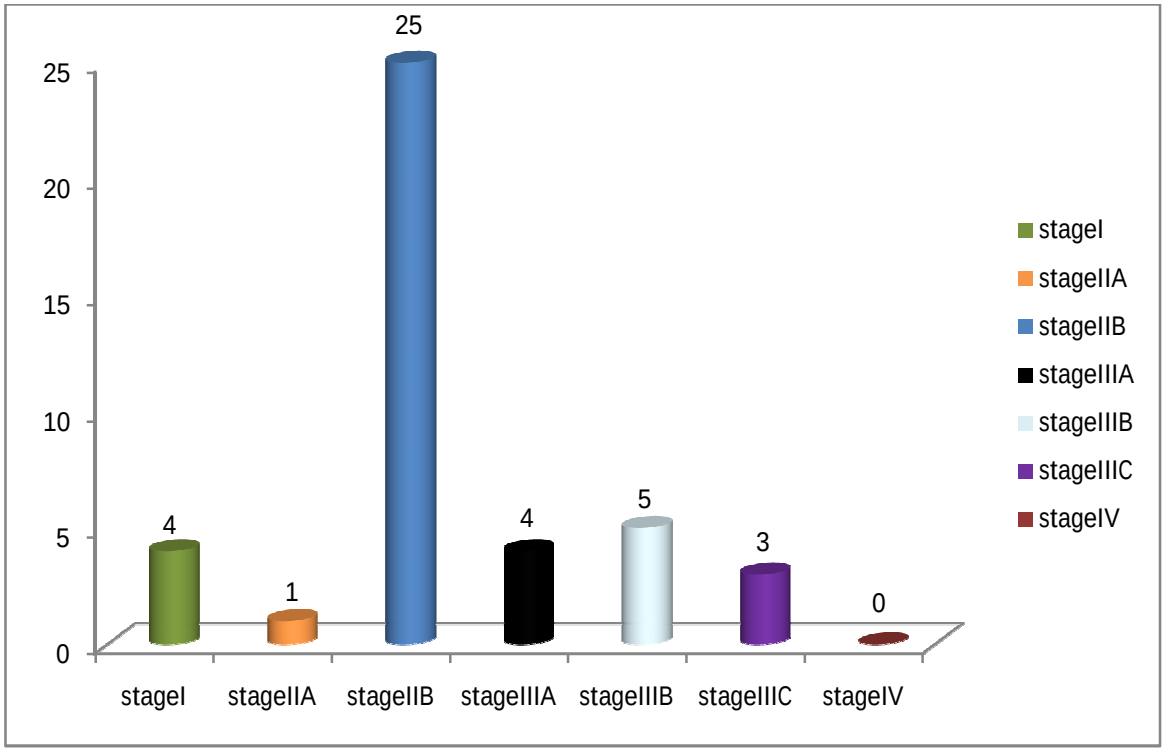
CARCINOMA BREAST AND HISTOLOGICAL GRADING



The above chart depicts that among 42 breast carcinoma cases taken for the study; exactly half of the cases belong to grade 2 histological type according to Nottingham modification of Scarff-Bloom-Richardson grading system.

CHART 4:

STAGING OF BREAST CANCER



The above chart shows among the 42 cases of breast cancer take up for study, nearly half the cases were found to be in stage II B according to the WHO TNM staging of breast cancer

TABLE 1:

NUMBER OF BREAST CARCINOMA CASES THAT ARE ER, PR AND HER 2 RECEPTOR POSTVE/NEGATIVE

EXPRESSION	**RECEPTORS**		
	ER	PR	HER 2
POSITIVE	20	16	29
NEGATIVE	22	26	13

The above table portrays that among the 42 cases of breast cancers taken for the study, almost one third of the cases are HER 2 receptor positive and half the cases for found to be Estrogen receptor positive

TABLE 2

NUMBER OF CASES WITH HISTOLOGICAL GRADING AND CORRELATION WITH ESTROGEN RECPETOR STATUS:

GRADE	ER POSITIVE	ER NEGATIVE	TOTAL NO. OF CASES
1	7	2	9
2	13	13	26
3	0	7	7
	20/42	22/42	42

The above table shows all the high grade tumors are ER negative and nearly all the low grade tumors are ER positive

TABLE 3

NUMBER OF CASES WITH DEGREE OF BRCA 1 EXPRESSION (MILD / MODERATE / STRONG / NO LOSS)

DEGREE OF BRCA 1 EXPRESSION	NUMBER OF CASES
LOSS OF EXPRESSION	0
MILD EXPRESSION	05
MODERATE EXPRESSION	03
STRONG EXPRESSION	34

The above table Shows 5/42 cases of carcinoma breast with reduced BRCA 1 expression

TABLE 4

NUMBER OF CASES WITH HISTOLOGICAL GRADING AND CORRELATION WITH BRCA 1 EXPRESSION

GRADE	CASES WITH LOSS OF BRCA 1 EXPRESSION	CASES WITH NORMAL BRCA 1 EXPRESSION	TOTAL NO. OF CASES
1	0	9	9
2	0	26	26
3	5	2	7
	5/42	37/42	42

The above table shows that out of seven high grade lesions five of the cases show reduced BRCA 1 expression, which implies that BRCA 1 mutated lesions are of higher in grade.

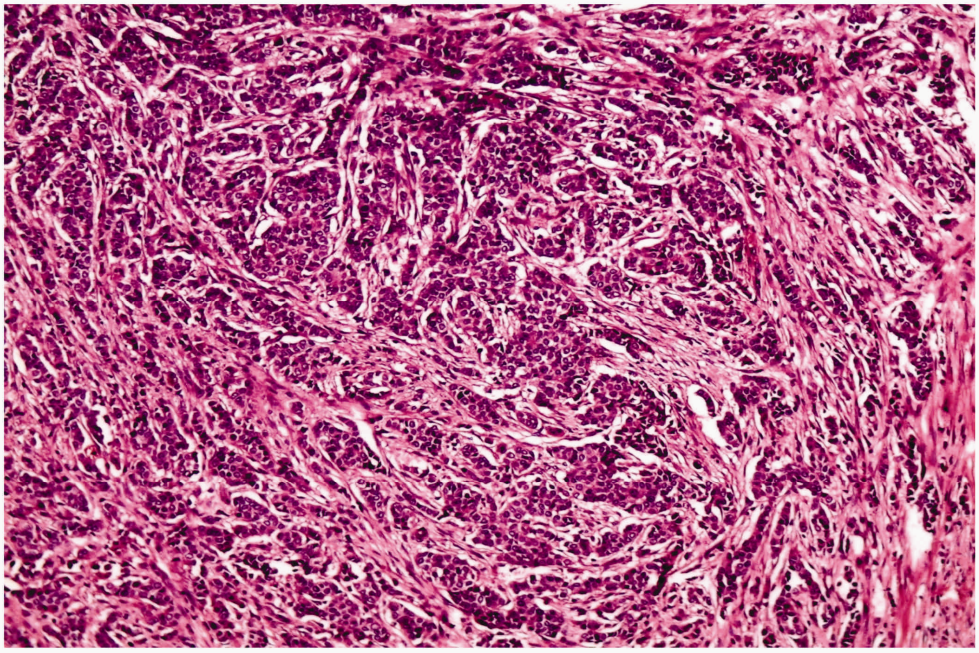


Fig 1 : Infiltrating Ductal Carcinoma (H&E, 400x)

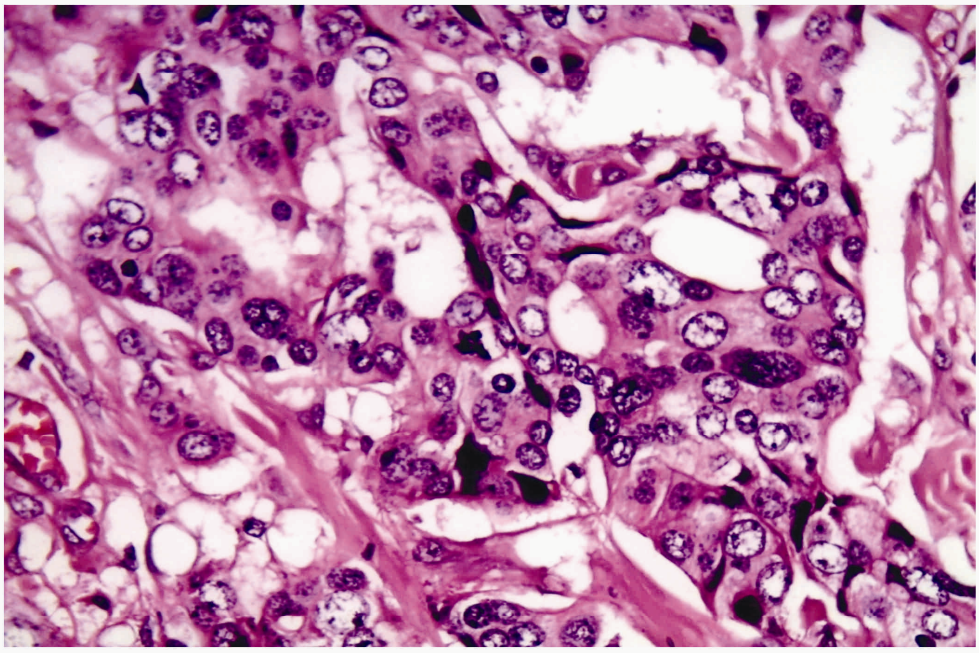


Fig 2 : Grade 3 IDC (H&E, 400x)

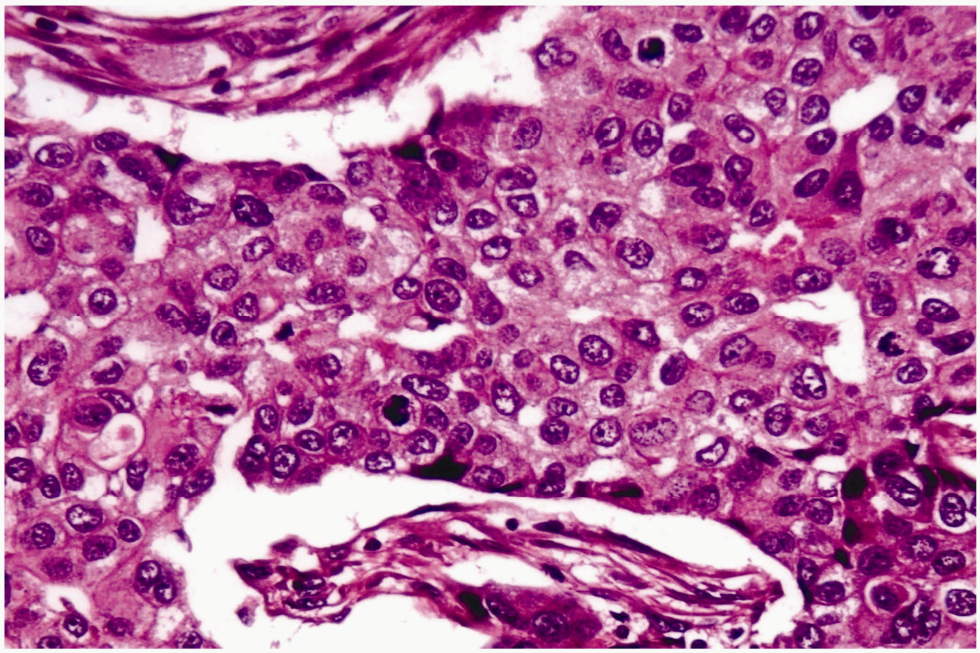


Fig 3 : Grade 3 IDC WITH MITOSIS
(H&E, 400x)

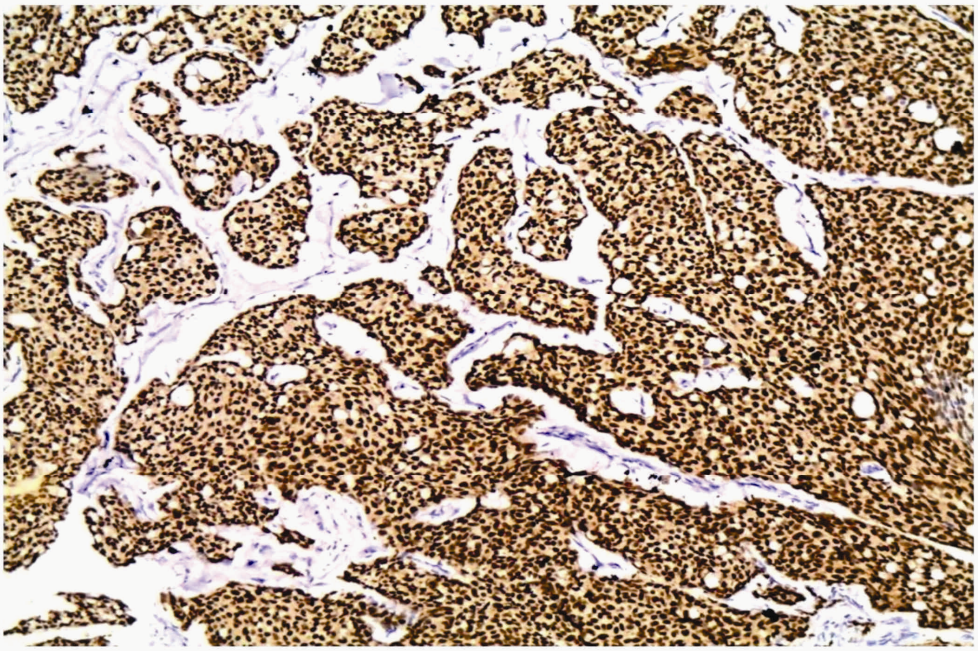


Fig 4 : IDC with ER nuclear positivity
(HC, 100x)

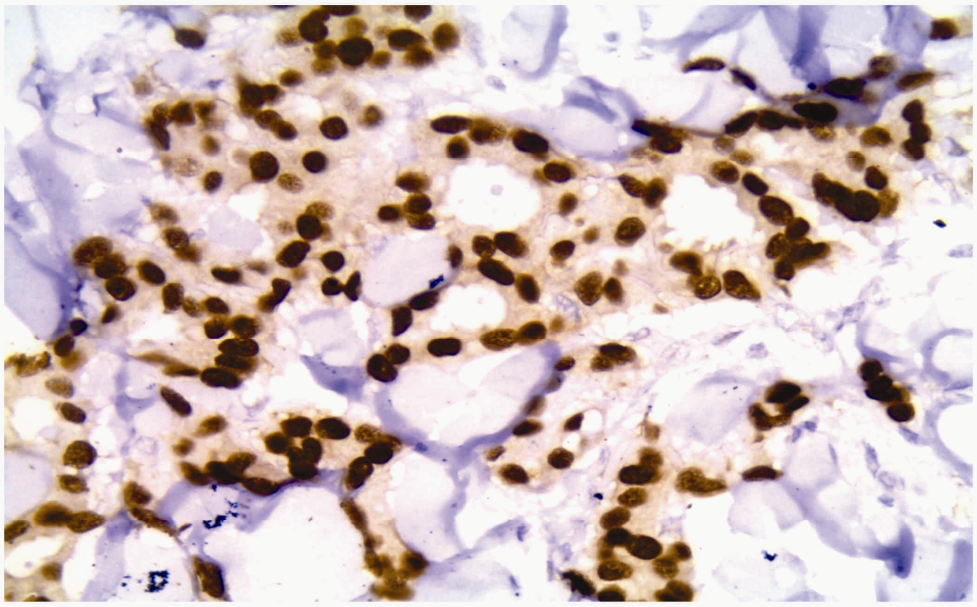


Fig 5 : IDC with nuclear positivity for PR (HC, 400x)

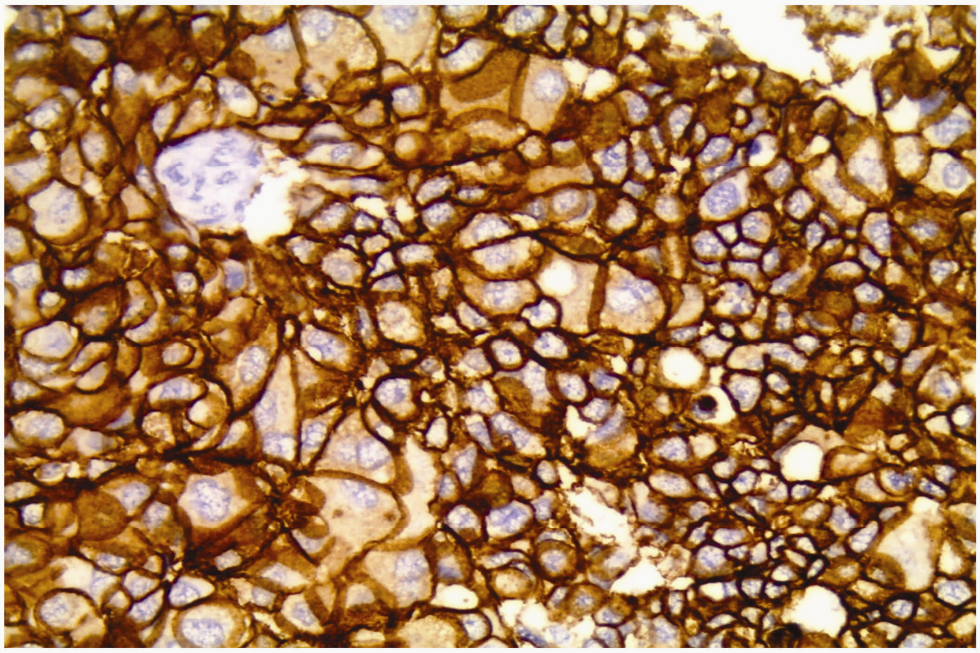


Fig 6 : IDC with complete strong membranous staining for HER 2 (IHC, 400x)

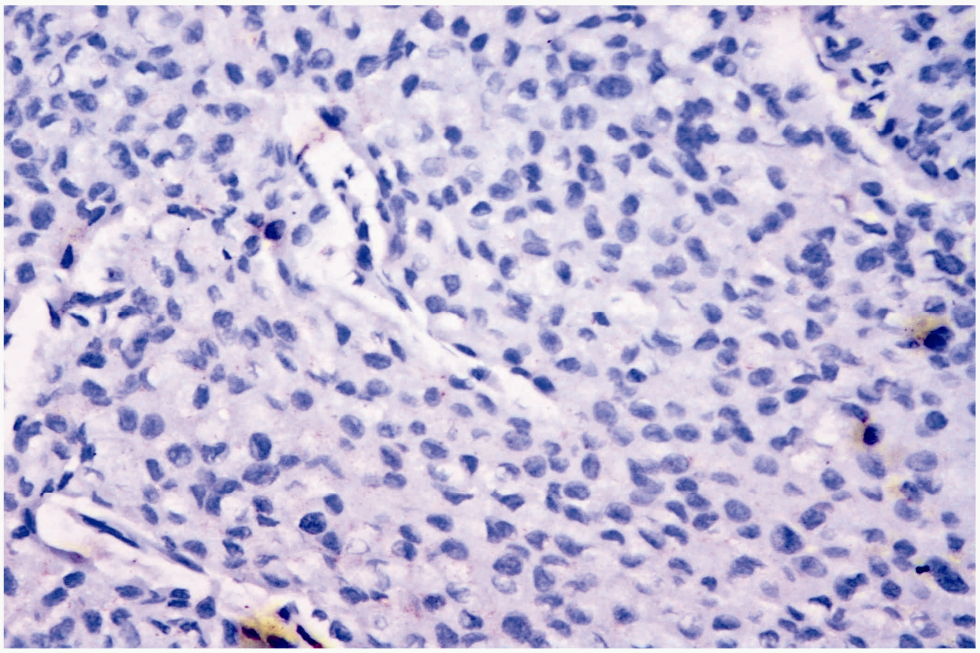


Fig 7 : IDC with loss of expression for BRCA 1 (IHC, 400x)

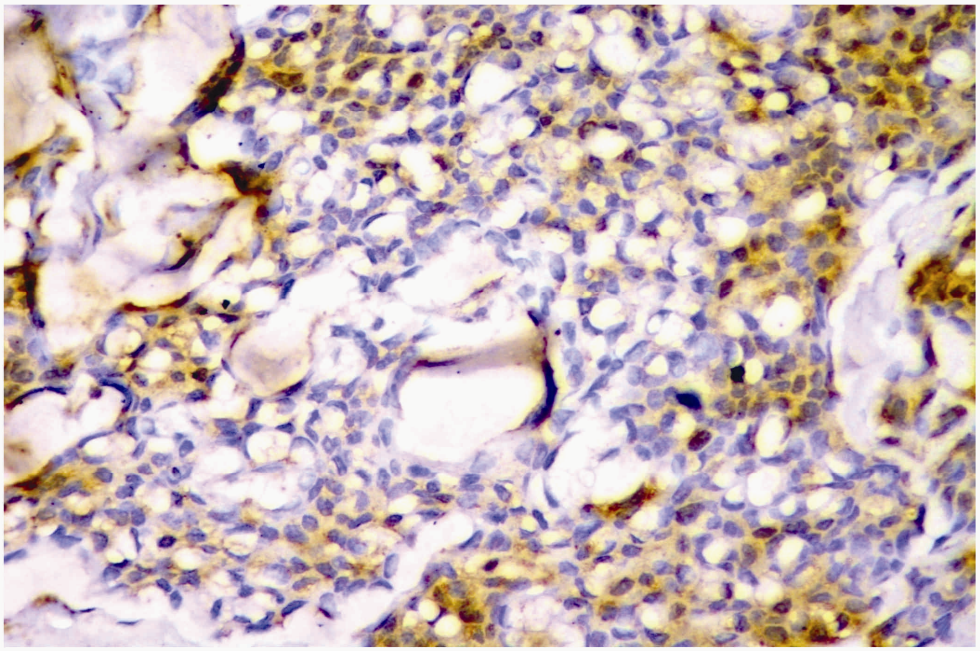


Fig 8 : IDC with moderate cytoplasmic expression for BRCA1 (IHC, 400x)

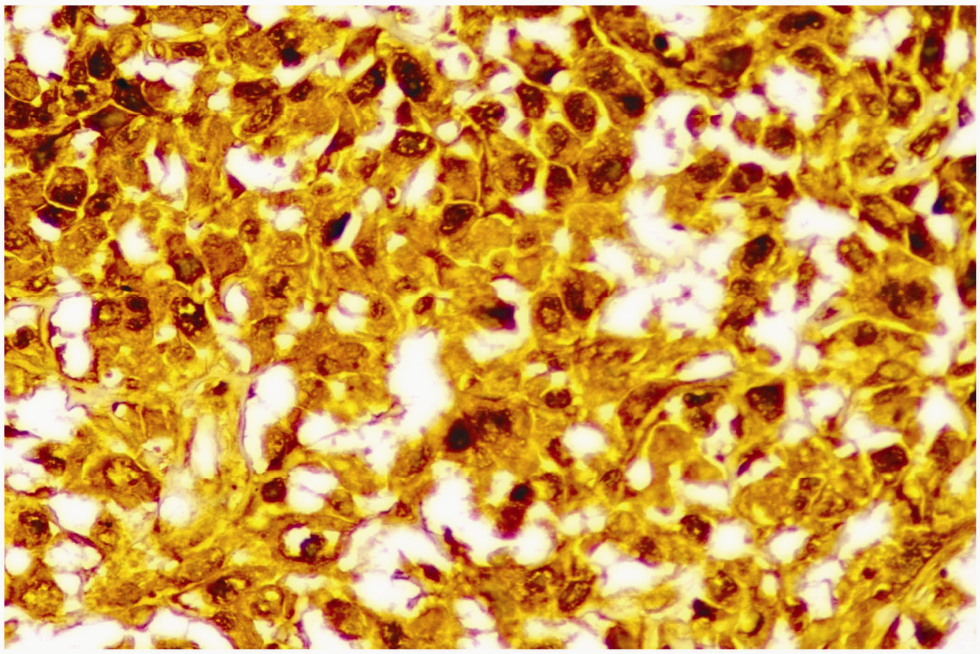


Fig 9 : IDC with strong nuclear and cytoplasmic expression for BRCA 1 (IHC, 400x)

RESULT ANALYSIS AND DISCUSSION:

Malignancies constituted 16.2 % among all biopsies received in the department of pathology of this institute and out of which 3.1 % is breast carcinoma. This incidence is somewhat less compared with the relative proportion of 26.07 % as reported in Chennai Cancer registry.[52]

Invasive breast cancer is the most common malignancy in women accounting for 22% of all female cancers and 26% of the cancers in affluent society.[10] This is more common in developed countries compared to that of underdeveloped countries

Invasive breast carcinoma cases taken in our study belong to the age group 21 to 80 years, with the mean age of 50.5 years (refer chart 1). Most of the studies involving carcinoma breast in women shows similar mean age of distribution.[47] Also the mean age of distribution is lower in developed countries compared to that of developing countries where there is a difference of around one decade.

The most common histological type of carcinoma breast in our study is infiltrating ductal carcinoma, not otherwise specified which accounts for about 83.3% (refer chart 2, figure 1). The value is similar to that of the study reported in literature were 78.58% of cases are of the type IDC,NOS[44]. This justifies the fact that infiltrating ductal carcinoma not otherwise specified occupies the largest group of infiltrating breast carcinomas according to the literature.

Our study portrays that most of the breast carcinoma cases detected, belong to grade 2 which accounts for about 61.9% followed by grade 1 which accounts for about 21.42% and grade 3 which accounts for about 16.66% (refer table 3) which is comparatively similar to that of the study conducted by Madhu Batra et al which reported the percentage of cases with grade 1 lesion as 15%, grade 2 lesion as 57.5% and grade 3 lesion as 27.5%.[45] Our study also highlighted that Stage II breast cancer accounts for about 61.9% of cases which is similar to the data obtained from a study which reported majority of the carcinoma breast cases (23-58%) belong to stage II lesion.[46](refer table 4).

Out of 42 cases taken in our study estrogen receptor was positive for 20 cases (47.61%) and negative for 22 cases (52.38%)(refer table 5, figure 4). The estrogen receptor positivity is quite high by 10% compared to that of the study reported in literature which showed only 35% of cases were estrogen receptor positive.[44,47] All high grade lesions are estrogen receptor negative (7/7) (100%) (Refer table 6). Out of these seven cases five cases showed loss of BRCA 1 expression which is significant.

Out of 42 cases HER 2 receptor was positive in 29 cases (69.04%) and progesterone receptor was positive in 16 cases (38.09%)(refer table 5, figure 5&6). They didn't show any correlation with grading or BRCA 1 expression which is similar to the study reported in literature.[48] HER 2 receptor expression is

high compared to that of study reported in literature and significantly high in comparison with the international values of 20-30%.[49] (refer table 5)

BRCA 1 a tumor suppressor gene when mutated can cause carcinoma breast, most of the mutations are germline mutations that are hereditary in preposition, whereas few of the cases can also have sporadic mutation due to hypermethylation of promotor region in BRCA 1gene. Germline mutation confers an estimated cumulative risk of breast cancer around 50% until age of 5 years.[50]

BRCA 1 mutated tumors are tumors of high grade and lack the expression of hormonal receptors. They can be aggressive tumors showing marked variation in histopathological features and thus influencing the prognosis of patients

Loss of BRCA 1 expression is considered in our study population when the cells show mild positivity for BRCA 1. Out of 42 cases 5 cases show loss of BRCA 1 expression which accounts for 11.9% (refer table 7, figure 7) which is in concordance with the study reported in literature.[45] Also among the high grade lesions (7 cases with grade 3 carcinoma), reduced BRCA 1 expression accounts for about 71.42% (5/7)(refer table 8). The value is similar to that of the study conducted by Fahd Al-Mulla et al, which reported that 77% of their cases with high grade lesion (grade 3) show reduced BRCA 1 expression.[51]Low expression of BRCA 1 is considered significant in our study, as the five cases which showed loss BRCA 1 expression were high grade tumors (grade 3) and were negative for estrogen receptor, which is similar to that reported in literature.[6]

SUMMARY AND CONCLUSION:

In conclusion, our study on breast carcinoma specimen revealed the incidence is around 3.1% of breast cancer among women. Majority of which occurs in the mean age group of 50.5 years. We infer that BRCA 1 mutation was seen mostly in grade 3 / high grade tumors. BRCA 1 mutation was also seen in hormone receptor negative cases, in particular estrogen receptors. The limitations of our study include small sample size. A larger prospective study on the utility of BRCA 1 immunohistochemistry is essential to prove the benefits of this mutation analysis for routine treatment and prognostic reasons.

BIBLIOGRAPHY

1. Kumar, Abbas, Faustor, Aster. *Robbins and Cotran Pathologic basis of disease*: South Asian Edition. Vol II: Elsevier; 2014
2. http://globocan.iarc.fr/old/factsheet.asp
3. Struewing JP, Hartge P, WacholderS, et al. The risk of cancer associated with specific mutations of BRCA 1 and BRCA 2 among Ashkenazi Jews. *N Engl J Med* 1997;336:1401-1408
4. Ford D, Easton DF, Stratton M, et al. Genetic hetrogenetiy and penetrance analysis of the BRCA 1 and BRCA 2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 1998;352:1337-1339
5. Hughes KS, Papa MZ, Whitney T, et al. Efficacy of bilateral prophylactic mastectomy and inherited predisposition to breast carcinoma. *Cancer* 1999;86:1682-1696
6. Rosen. *Rosen's Breast Pathology*, 4th edition. Philadelphia: Lipincott Williams & Wilkins; 2014
7. Inderbir Singh. *Human Embryology*, 9th edition: Macmillan;2012
8. Susan. *Gray's Anatomy*, 4th edition. Churchill Livingstone:Elsevier; 2009
9. Rosai. *Rosai and Ackermann's Surgical Pathology*, 10th edition Edinburgh: Mosby; 2011
10. Fattaneh A. Tavassoli& Peter Devilee. *Pathology and Genetics of Tumors of the Breast and Female Genital Organs*, 3rd ed. Lyon: IARC Press; 2003

11. Hassiotou F, Geddes D. Anatomy of the human mammary gland: current status of knowledge. *Clinical Anatomy* 2013;26:29-48

12. R. Ferla, V. Calo, S Cascio1 et al. Founder mutations in BRCA1 and BRCA2 genes. *Annals of Oncology* 18, 2007; vi93–vi98

13. Wang DY et al. Influence of reproductive history of age at diagnosis of breast cancer and prognosis. *Int J Cancer* 1985; 36:427-432.

14. Ross RK, Paganini-Hill A, Wan PC, Pike MC: Effect of hormone replacement therapy on breast cancer risk estrogen versus estrogen plus progestin. *J Natl Cancer Inst* 2000; 92:328-332.

15. Goss PE, Sierra S: Current perspectives on radiation-induced breast cancer. *J ClinOncol* 1998; 16:338-347.

16. Wooster R, Weber BL: Breast and ovarian cancer. *N Engl J Med* 2003; 348:2339-2347.

17. Tan DS, Marchio C, Reis-Filho JS: Hereditary breast cancer: from molecular pathology to tailored therapies. *J ClinPathol* 2008; 61:1073-1082.

18. Gage M, Wattendrof D, Henry LR. Translational advances regarding hereditary breast cancer syndromes. *J SurgOncol* 2012; 105:444-451

19. Schnitt SJ et al. Pathologic findings on re-excision of the primary site in breast cancer patients considered for treatment by primary radiation therapy. *Cancer* 1987; 59:675-681.

20. Lakhani SR et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA 1 & BRCA 2 mutations. *J Natl Cancer Inst* 1998; 90:1138-1145

21. Ellis IO et al. Pathological prognostic factors in breast cancer II. Histological type. Relationship with survival in a large study with long term follow- up. *Histopathology* 1992; 20:479-489

22. Carstens PH et al. Tubular carcinoma of the breast : A clinicopathologic study of 35 cases. *Am J ClinPathol* 1972; 58:231-238

23. Armes JE, Venter DJ: The pathology of inherited breast cancer. *Pathology* 2002; 34:309-314.

24. SapinoA et al. Expression of apocrine differentiation markers in neuroendocrine breast carcinomas of aged women. *Mod Pathol* 2001; 14:768-776

25. Nassar H et al. Clinicopathologic analysis of invasive micropapillary differentiation in breast carcinoma. *Mod Pathol* 2001; 14:836-841

26. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. *Breast Cancer Research & Treatment* 1992; 22(3):207-219Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC Cancer Staging Manual and the Future of TNM. *Ann SurgOncol* 2010; 17:1471-1474

27. Lagios MD, Westdahl PR, Margolin FR, Rose MR: Duct carcinoma in situ. Relationship of extent of noninvasive disease to the frequency of occult invasion, multicentricity, lymph node metastases, and short-term treatment failures. *Cancer* 1982; 50:1309-1314.

28. Davis BW, Gelber R, Goldhirsch A, Hartmann WH, Hollaway L, Russell I, Rudensta CM: Prognostic significance of peritumoral vessel invasion in clinical trials of adjuvant therapy for breast cancer with axillary lymph node metastasis. *Hum Pathol* 1985; 16:1212-1218.

29. Adami H-O, Malker B, Holmberg L, Persson I, Stone B: The relation between survival and age at diagnosis in breast cancer. *N Engl J Med* 1986; 315:559-563.

30. Reed W, Sandstad B, Holm R, Nesland JM: The prognostic impact of hormone receptors and c-erbB-2 in pregnancy-associated breast cancer and their correlation with BRCA1 and cell cycle modulators. *Int J SurgPathol* 2003; 11:485-488.

31. American Joint Committee on Cancer: *AJCC cancer staging manual*, edition 7, Newyork: Springer; 2009

32. Nadji M, Gomez-Fernandez C, Ganjei-Azar P, et al. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J ClinPathol* 2005;123:21–27.

33. Goldstein NS, Ferkowicz M, Odish E, et al. Minimum formalin fixation time for consistent estrogen receptor immunohistochemical staining of invasive breast carcinoma. *Am J ClinPathol* 2003;120:86–92

34. Allen M Gown. Current issues in ER and HER2 testing by IHC in breast cancer. *Modern Pathology* 2008; 21, S8–S15

35. Allred DC, Harvey JM, Berardo M, et al. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998;11:155–168.

36. Harvey JM, Clark GM, Osborne CK, etal.Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assays for predicting response to adjuvant therapy in breast cancer. *J ClinOncol* 1999;17:1474–85.

37. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J ClinOncol* 2007;25:118–145

38. W-Y Lee, Y-T Jin, T-W Chang et al. Immunolocalization of BRCA 1 protein in normal breast tissue and sporadic invasive ductal carcinomas: A correlation with other biological parameters. *Histopathology* 1999; 34:106-112

39. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. *J Natl Cancer Inst* 2001;93:1633–1637.

40. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med* 2002;346:1609–1615.

41. Haffty BG, Harold E, Khan AJ, et al. Outcome of conservatively managed early-onset breast cancer by BRCA 1/2 status. *Lancet* 2002;359: 1471–1477.

42. Taylor RC, Chanrong, BJ NAney, Wu N. Techniques of immunohistochemistry: principles, pitfalls and standardization. In: David J Dabbs (ed). *Diagnostic immunohistochemistry*. 1st edition. Philadelphia, Churchill Livingstone 2002; 3-34.

43. Suvarna K, Layton C, Bancroft J. *Bancroft's Theory and Practice of Histological Techniques*, 6thed: Churchill-Livingstone;2007

44. Maria Comanescu, Carmen Florina Popescu. BRCA1 expression in invasive breast carcinomas and clinicopathological correlations. *Romanian Journal of Morphology and Embryology* 2009; 50(3):419–424

45. MadhuBatra et al. Expression of BRCA 1 & BRCA 2 proteins and their correlation with clinical staging in breast cancer. *Journal of Cancer Research and Therapeutics* 2015; 11:158-163

46. Nair MK, Varghese C, Swaminathan A. Current scenario intervention strategies and projections for 2015. *Burden of disease in India,* 2005; 218-225

47. Manisha Sharma, MriduManjari, MohitMadhukar, Manas Madan &Gazalpreet Kaur Sra. Comparison of Clinicopathological Characteristics of BRCA1 Associated Breast Cancer Cases with BRCA1 Negative Breast Cancer Cases: A Prospective Study of 100 Women in a Tertiary Care Cancer Hospital Global Journal of Medical Research. *Global Journals Inc*.(US) 2014;15:22-24

48. AnsquerYetal. Expressionof BRCA 1,HER 1 and HER 2 in sporadic breastcancer and relationships to other clinicopathological prognostic features. *Anticancer Rest.* 2005;25:4535-39.

49. ElaheKeyhani, AhadMuhammadnejad, MasoudKarimlou. Prevalence of HER 2 positive Invasive breast cancer : A systemic review form Iran. *Asian Pacific J Cancer Prev* 2012;13:5477-5482

50. KiyotsuguYoshikawaetal. Reduction of BRCA1 Protein Expression in Japanese Sporadic Breast Carcinomas and Its Frequent Loss in BRCA1-associated Cases. *Clinical Cancer Research* 1999;5:1249–1261

51. Fahd Al-Mulla, MaheraAbdulrahman, GovindarajuluVaradharaj, Nadeem Akhter, Jehoram T. Anime. BRCA1 Gene Expression in Breast Cancer: A Correlative Study between Real-time RT-PCR and Immunohistochemistry.

52. Journal of Histochemistry&Cytochemistry 2005;53:621-629.

S.NO	SLIDE NO	AGE/SEX	Histological type of tumor	size-cm	No. of nodes	Grade	TNM Staging	Staging	ER	PR	HER 2	BRCA 1 Expression
1	4265/12	47/F	Infiltarting ductal ca, NOS	3.5	0/11	2	T2N0M0	II A	NEGATIVE	NEGATIVE	POSITIVE	STRONG
2	4049/12	51/F	IDC, NOS	3.7	0/7	3	T2N0M0	II A	NEGATIVE	NEGATIVE	POSITIVE	LOSS
3	1187/13	47/F	IDC, NOS	16	0/20	3	T4N0M0	III B	NEGATIVE	NEGATIVE	POSITIVE	STRONG
4	2000/13	60/F	IDC, NOS	2	1 of 8	2	T1CN1M0	II A	POSITIVE	POSITIVE	POSITIVE	STRONG
5	2166/13	42/F	IDC, NOS	4	0/15	3	T2N0M0	II A	NEGATIVE	NEGATIVE	POSITIVE	LOSS
6	3262/13	68/F	IDC, NOS	4.5	0/7	1	T2N0M0	II A	POSITIVE	POSITIVE	POSITIVE	STRONG
7	4310/13	58/F	IDC, NOS	2	0/11	1	T1CN0M0	IA	NEGATIVE	NEGATIVE	POSITIVE	STRONG
8	4614/13	65/F	IDC, NOS	4	6 of 18	2	T2N2M0	IIIA	NEGATIVE	NEGATIVE	POSITIVE	STRONG
9	5324/13	32/F	IDC, NOS	6	0/16	2	T3N0M0	IIB	POSITIVE	POSITIVE	POSITIVE	STRONG
10	3600/13	52/F	IDC, NOS	9	11 of 19	3	T3N3M0	IIIC	NEGATIVE	NEGATIVE	POSITIVE	STRONG
11	3704/13	40/F	IDC, NOS	4	1 of 11	2	T2N1M0	IIB	NEGATIVE	NEGATIVE	NEGATIVE	STRONG
12	3415/14	55/F	IDC, NOS	3	0/27	3	T2N0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	MODERATE
13	533/14	53/F	IDC, NOS	7.5	1 of 19	2	T3N1M0	IIIA	NEGATIVE	NEGATIVE	NEGATIVE	STRONG
14	135/14	65/F	Invasive Lobular Carcinoma	3.5	0/12	2	T2N0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	STRONG
15	2363/14	62/F	IDC, NOS	8	0/17	2	T3N0M0	IIB	POSITIVE	POSITIVE	NEGATIVE	STRONG
16	1535/14	75/F	Invasive Apocrine Carcinoma	4	0/9	2	T2N0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	STRONG
17	1159/14	38/F	Invasive Apocrine Carcinoma	3.5	0/15	2	T2N0M0	IIA	POSITIVE	POSITIVE	POSITIVE	MODERATE
18	4896/14	45/F	IDC, NOS	2.5	0/15	2	T2N0M0	IIA	POSITIVE	POSITIVE	POSITIVE	STRONG
19	4736/14	52/F	IDC, NOS	2	0/7	3	T4N2M0	IIIB	NEGATIVE	NEGATIVE	NEGATIVE	LOSS
20	4293/14	60/F	IDC, NOS	3.5	0/11	2	T1CN0M0	IA	POSITIVE	POSITIVE	POSITIVE	STRONG
21	393/14	42/F	IDC, NOS	2	0/25	1	T2N0M0	IIA	POSITIVE	POSITIVE	POSITIVE	STRONG
22	4036/14	74/F	Mucinous Carcinoma	9	0/9	1	T1CN0M0	IA	NEGATIVE	NEGATIVE	NEGATIVE	STRONG
23	2958/14	63/F	IDC AND Mucinous carcinoma	8	5 of 13	2	T3N1M0	IIIA	POSITIVE	POSITIVE	NEGATIVE	STRONG
24	5069/14	77/F	IDC, NOS	3.5	1 of 10	1	T2N1M0	IIB	POSITIVE	POSITIVE	NEGATIVE	STRONG
25	627/15	34/F	IDC, NOS	2.5	13 of 30	2	T2N3M0	IIIC	NEGATIVE	NEGATIVE	NEGATIVE	STRONG
26	932/15	50/F	IDC, NOS	3	1 of 15	2	T3N1M0	IIIA	POSITIVE	NEGATIVE	POSITIVE	STRONG

27	4170/12	62/F	IDC, NOS	4.5	0/31	2	T3N0M0	IIB	NEGATIVE	NEGATIVE	POSITIVE	STRONG
28	4116/12	53/F	IDC, NOS	5	1 of 17	1	T2N1M0	IIB	POSITIVE	POSITIVE	POSITIVE	STRONG
29	2577/12	40/F	IDC, NOS	2.5	8 of 25	2	T1N2M0	IIIA	POSITIVE	POSITIVE	POSITIVE	STRONG
30	3675/13	64/F	IDC, NOS	1	5 of 10	2	T1bN2M0	IIIA	POSITIVE	POSITIVE	POSITIVE	MODERATE
31	3279/12	66/F	Invasive Papillary Carcinoma	5	0/13	1	T2N0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	STRONG
32	4254/14	68/F	IDC With Apocrine changes	3	25 of 32	2	T2N3M0	IIIC	NEGATIVE	NEGATIVE	NEGATIVE	STRONG
33	3840/13	47/F	IDC, NOS	3	1 of 19	2	T2N1M0	IIB	POSITIVE	POSITIVE	POSITIVE	STRONG
34	1016/14	48/F	IDC, NOS	3.5	3 of 22	3	T2N1M0	IIB	NEGATIVE	NEGATIVE	POSITIVE	STRONG
35	4013/14	56/F	IDC, NOS	2.5	0/23	3	T2N0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	LOSS
36	1012/13	47/F	IDC, NOS	2.5	6 of 15	2	T2N0M0	IIA	POSITIVE	POSITIVE	POSITIVE	STRONG
37	2708/12	44/7	IDC, NOS	2	0/16	2	T1cN0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	STRONG
38	807/12	48/F	IDC, NOS	2.5	0/19	2	T2N0M0	IIA	POSITIVE	POSITIVE	NEGATIVE	STRONG
39	5650/14	45/F	IDC, NOS	4.5	1 of 13	2	T2N1M0	IIB	POSITIVE	POSITIVE	NEGATIVE	STRONG
40	325/14	33/F	IDC, NOS	2	0/11	1	T1cN0M0	IIA	POSITIVE	NEGATIVE	NEGATIVE	STRONG
41	3415/14	55/F	IDC, NOS	3	0/8	3	T2N0M0	IIA	NEGATIVE	NEGATIVE	POSITIVE	LOSS
42	2362/14	62/F	IDC, NOS	8	0/13	2	T3N0M0	IIB	POSITIVE	POSITIVE	POSITIVE	STRONG